A Summary of Current Program, 7/1/65

and Preliminary Report of Progress

for 7/1/64 to 6/30/65

ANIMAL DISEASE AND PARASITE RESEARCH DIVISION of the AGRICULTURAL RESEARCH SERVICE UNITED STATES DEPARTMENT OF AGRICULTURE and related work of the STATE AGRICULTURAL EXPERIMENT STATIONS

This progress report is primarily a tool for use of scientists and administrators in program coordination, development and evaluation, and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of progress on USDA and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of USDA and cooperative research issued between July 1, 1964, and June 30, 1965. Current agricultural research findings are also published in the monthly USDA publication, Agricultural Research. This progress report was compiled in the Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland.

UNITED STATES DEPARTMENT OF AGRICULTURE

Washington, D. C.

July 1, 1965

TABLE OF CONTENTS

INTRODUCTION

The Animal Disease and Parasite Research Division administers a national program of basic and applied research on diseases of cattle, poultry, swine, sheep, horses, and fur-bearing animals. The Division consists of three large laboratories and eleven smaller, specialized laboratories. The large ones are the Beltsville Parasitological Laboratory, the National Animal Disease Laboratory at Ames, Iowa, and the Plum Island Animal Disease Laboratory at Greenport, Long Island, New York. The research at these locations cover, respectively, animal parasites, animal diseases existing in the United States, and foreign animal diseases. The smaller, specialized laboratories are located as follows:

The Southeast Poultry Research Laboratory, Athens, Georgia.
The Regional Animal Parasite Laboratory, Auburn, Alabama, with substations at Experiment, Georgia, and State College, Mississippi.
The Animal Disease Research Laboratory, Pullman, Washington.
Toxicological Research Laboratory, Kerrville, Texas.
Sheep Disease Research Laboratory, Denver, Colorado.
Poisonous Plants Research Laboratory, Logan, Utah
Parasite Research Laboratory, Albuquerque, New Mexico
Parasite Research Laboratory, University Park, New Mexico.
Parasite Research Laboratory, Tifton, Georgia
Cooperative Research at the State Veterinary Research Institute, Amsterdam, Holland.
Cooperative Research at the East African Veterinary Research Organization, Kabete, Kikuyu, Kenya, East Africa.

In addition, the Division engages in cooperative research involving sixty cooperative projects at various Universities and State Experiment Stations. The Division's research program is coordinated by the Office of the Director, located at Beltsville, Maryland.

The Animal Disease and Parasite Research Division has contributed many significant research findings aimed at reducing the heavy losses to the livestock industry resulting from animal diseases. Several of these research discoveries have accounted for savings to the livestock industry in excess of the total cost of animal disease research in the U. S. Department of Agriculture since the inception of the Bureau of Animal Industry in 1887. Among these discoveries are the isolation and description of the genus of bacteria known as Salmonella; the role of arthropod vectors in spreading infectious diseases; the cause of hog cholera and the development of the first immunization procedure for this disease; the first successful treatment for hookworms in animals and man; the development of Strain 19 vaccine to prevent brucellosis, and the discovery of the cause of hyperkeratosis in cattle. Some of the more recent accomplishments by this Division are -

Studies with tissue culture-modified rinderpest virus as an immunizing agent. Through serial passage of a modified strain of rinderpest virus in tissue culture by the limiting dilution technique, an avirulent form of rinderpest virus resulted. This modified virus, when inoculated into cattle, resulted in a high degree of immunity and protection against the disease. Three weeks after immunization cattle challenged with 100,000 lethal doses of virulent rinderpest virus showed no signs of illness. Subsequent to this work, officials in Egypt requested this modified rinderpest virus immunizing agent for use in their country. Results to date have been very good in water buffalo, native Egyptian cattle and cattle imported into Egypt.

Pulmonary adenomatosis has been produced in cattle with the oxides of nitrogen. The pulmonary lesions produced by the inhalation of the oxides of nitrogen are similar to those observed in man affected with silo-fillers disease. Information from this research will be useful in explaining the pathogenesis of pulmonary alterations produced in both cattle and men exposed to these toxic gases. It is expected that this study will explain some of the alterations observed in bovine emphysema, bovine asthma, and fog disease.

Ultraviolet radiation and Moraxella bovis work together to cause bovine pink-eye. In a series of preliminary experiments, a mercury sunlamp was found to enhance the effect of Moraxella bovis infection upon the bovine eye. The resulting disease was indistinguishable from field cases of infectious bovine keratoconjunctivitis (pink-eye). This method makes possible the study of the disease under controlled conditions at any time of year. The investigators propose that ultraviolet has a primary etiological role in the disease.

The pathogenesis of brucellosis in male swine studied. Sexually mature boars were exposed to Brucella suis and were killed at intervals after exposure to determine the pathogenesis of brucellosis in boars. Clinical signs were observed, serologic and bacteriologic studies of blood samples were conducted, and tissues were thoroughly examined at necropsy for pathologic and bacteriologic evidence of infection. The period in which isolations of Br. suis were most frequently made and histopathologic alterations most often observed extended from 2 through 6 weeks post-exposure. Brucella suis was isolated most frequently from lymph nodes and accessory genital glands. Gross pathologic alterations were confined to seminal vesicles and their regional lymph nodes. Histopathologic alterations were observed most frequently in lymph nodes, accessory genital glands, livers, and bones.

Carrier swine chronically affected by leptospirosis cured with antibiotics. Leptospires were eradicated from the kidneys of carrier swine by injections of streptomycin. The method will be of value in the control of leptospirosis in domestic animals. Although the treatment requires subcutaneous

injections at the present time, investigators at the National Animal Disease Laboratory are searching for drugs which can be administered in the feed or drinking water.

Immunizing antigens of Pasteurella multocida. Particulate antigens were isolated from two distinct immunogenic strains of Pasteurella multocida of avian origin. An emulsified vaccine prepared with antigens from one of the strains induced 100% immunity in chickens to an homologous challenge which killed 100% of the unvaccinated controls. The antigens possessed many of the chemical and physical properties ascribed to endotoxins. Injections of fractional milligram amounts of the antigens into chickens produced signs which are usually observed in acute cases of fowl cholera, such as depression, increased salivation, diarrhea, and some times death.

Two viruses may be associated with transmissible gastroenteritis of pigs. Continuing research on transmissible gastroenteritis (TGE) of swine has indicated that there are two viruses which may be associated with TGE. One virus, a cytopathic virus, has been isolated from the intestinal tissue from several outbreaks of TGE. Antibodies against this virus are present in some of the convalescent sera from outbreaks of TGE in the field. Characterization studies indicate that the cytopathic virus belongs in the myxo class of viruses. The noncytopathic virus has been isolated from one outbreak of TGE from which the cytopathic virus could not be isolated, and from one outbreak of TGE from which the cytopathic virus had already been isolated. There is no apparent cross protection nor cross neutralization between the two viruses. Either virus can produce vomiting and diarrhea in newborn pigs. The role and relationship that each of these two viruses play in the overall disease, the problem of laboratory diagnosis, and the control and treatment of TGE will be elucidated by further research.

Causal agent (Anaplasma marginale) of bovine anaplasmosis in the United States, comparable in virulence to supposedly benign form (Anaplasma centrale) in Africa. Bovine anaplasmosis, a costly disease, is caused by a microscopic parasite, Anaplasma marginale, located marginally in red blood cells. A related form, Anaplasma centrale, located centrally in red blood cells, occurs in African cattle. The latter is said to be harmless, and to protect against anaplasmosis caused by the "marginal" form. Critical comparisons of these two forms were made experimentally in Africa by a Beltsville scientist, using a "marginal" form transported to Africa, a "central" form procured there, and susceptible, imported cattle. Significant differences between the two parasites were not detected, either in disease produced, including degree of anemia, or immunization ability. The findings of this evaluation are important because of strong recommendations that Anaplasma centrale be brought into the United States and used as a vaccine against anaplasmosis caused by the indigenous parasite. To do so could result in another disease agent becoming established in American cattle.

Destruction of trichinae by freezing at 0°F. delayed by previous exposure to near-freezing temperatures. Research at the Beltsville Parasitological Laboratory on the effects of cold temperatures on trichinae indicate that prolonged exposure to 34°F. increases the capacity of trichinae in fresh pork to resist destruction by freezing. One-pound patties of fresh ground pork, containing 25,000 to 270,000 trichinae per pound, were precooled to 34°F. for 51 and 135 days, respectively, immediately before being exposed to 0°F. in a home freezer of 9 cubic feet capacity. Trichinae in pork precooled to this temperature for the periods indicated survived exposure to 0°F. for 89 to 144 hours, respectively, whereas, trichinae in patties not so treated lived only 72 hours. These results show that the temperatures at which trichinous pork is held prior to freezing may influence the time required to kill them by this method.

Another new and probably widespread human disease is born of Beltsville competence. An entire worm, and parts of another worm, in tissue sections recovered from the brain of a mental patient in Hawaii were received for identification. The worms were determined to be the rodent lungworm, Angiostrongylus cantonensis. Based on this identification and subsequent findings by public health workers, the rodent lungworm is now believed responsible for eosinophilic meningoencephalitis of man. This disease, previously of unknown etiology, occurs in Hawaii, Tahiti, and other South Pacific Islands. Man probably becomes infected by eating mollusks which serve as the intermediate host of the parasite.

The new world hookworm of man was among the first of a long series of human parasites discovered by Department parasitologists.

The persistence of mammalian viruses and rickettsiae studied in endoparasites. Silver salmon infected with the metacercariae of the "salmon poisoning" fluke (Nanophyetus salmincola)remain infected after they have migrated to sea. Metacercariae remain viable in such salmon for at least 5 years. These metacercariae are capable of transmitting salmon disease (Neorickettsia helminthoeca) and another closely related agent (Elokomin fluke fever) to susceptible dogs throughout this period. Elokomin fluke fever is a disease closely resembling infectious mononucleosis of man with an increase in mononuclear cells, a rise in titer to the heterophile antibody, and a generalized lymphadenopathy. It is distinct from Neorickettsia helminthoeca on cross protection, serum neutralization and complement fixation tests.

Bluetongue virus has been discovered and photographed in the cells of the salivary gland of Culicoides variipennis, a known vector of BT disease. C. variipennis (gnat) has been infected with BT virus and the salivary glands dissected free of the insect and studied in the electron microscope. The method of replication of the virus in the salivary glands cells is being established. This basic piece of research is the first successful ultracytopathological study of an arbovirus in insect cells. Knowledge of this kind is essential to an understanding and control of arboviruses that affect man and animals.

AREA NO. 1 - INFECTIOUS AND NON-INFECTIOUS DISEASES OF CATTLE

Problem. Losses from infectious and non-infectious diseases of cattle, other than those due to parasites, are estimated at approximately $600 million annually. These losses materially increase costs of production and conversely decrease profits. In turn, they contribute to the cost of every purchase of meat, milk, and other cattle products to the consumer. Some of these diseases are transmissible to man. Determination and definition of the causes of cattle diseases, explorations for efficient methods of diagnosis, prevention, control, and when feasible, eradication, are the purposes of the research program.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving biochemists, microbiologists, pathologists, and veterinarians engaged in both basic studies and the application of known principles to the solution of infectious and non-infectious diseases of cattle. Research is being conducted on the diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 29.0 professional man-years. This effort is divided among sub-headings as follows:

Brucellosis of Cattle 2.5 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the University of Minnesota, the University of Wisconsin, and with the Ohio Agricultural Experiment Station. A project on the immunizing effect of Brucella cell wall is in progress at the Hebrew University, Jerusalem, Israel, under a PL 480 Grant of funds equivalent to $31,950.00 over a 3-year period.

Vibriosis of Cattle 2.0 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the New York State Veterinary College at Ithaca.

Tuberculosis of Cattle 2.0 at the National Animal Disease Laboratory, Ames, Iowa, and through a contract with the Michigan State University at East Lansing.

Mucosal-Respiratory Disease-Complex 3.5 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the Colorado State University at Fort Collins, the Agricultural Experiment Station, Purdue University at Lafayette, Indiana, and the Iowa State University, Ames.

Mastitis of Cattle 3.5 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the University of California, Davis.

Respiratory Disease of Cattle (Shipping Fever) 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

Infertility in Cattle, other than Vibriosis and Trichomoniasis 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

Epizootic Bovine Abortion 0.5 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the University of California, Davis.

Foot Rot (Infectious Pododermatitis) of Cattle 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Etiological, Cytological and Histochemical Studies of Pulmonary Adenomatosis in Cattle 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Immunization against Bovine Leptospirosis 1.5 at the National Animal Disease Laboratory, Ames, Iowa.

Chemotherapy in Leptospirosis 1.5 at the National Animal Disease Laboratory, Ames, Iowa.

Enteritis of Young Calves 0.5 at the National Animal Disease Laboratory, Ames, Iowa, and under a contract with the University of Idaho, Moscow.

Bovine Lymphosarcoma 1.5 at the National Animal Disease Laboratory, Ames, Iowa.

Paratuberculosis of Cattle (Johne's Disease) 2.0 at the National Animal Disease Laboratory, Ames, Iowa

Keratitis (Pink Eye) 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

PROGRAM OF STATE EXPERIMENT STATIONS

The State experiment stations are devoting an increasing amount of effort toward research directed at the prevention, control and eradication of cattle diseases. The objectives of these studies are concerned not only with more efficient production of meat and milk, but also with the production of products which are wholesome and safe for human consumption.

Many of the Western states are cooperating (W-88, Enteric Diseases of Neonatal Calves) in initiating basic and applied studies to determine the cause and control of intestinal infections in calves.

Increasing interest and support is being given to the cause and control of bovine leukosis. Workers also seek basic information concerning the possible relationship of this disease to cancer in man and other animals.

Cooperative regional studies, among the Southern (S-30, Diseases of Reproduction) and Northeastern states (NE-40, Pathology of Breeding Failure), seek to determine the relation of various infectious agents to poor reproductive performance and sterility in cattle. Increasing attention is being directed toward the role of viruses in infertility. A new vaccine against vibriosis offers promise. However, antigenic variation in strains of the organism causes continued concern to workers. The role of leptospira in infertility is being determined. Basic studies pertaining to the diagnosis and pathogenicity of the different serotypes of the organism continues to receive considerable attention.

The North Central states are cooperating (NCR-29, Shipping Fever of Cattle; NCR-37, Mucosal Disease of Cattle) to determine the inter-relationships between various agents and factors associated with respiratory infections in cattle. The relation of virus diarrhea and infectious bovine rhinotracheitis to the respiratory disease complex is being given considerable attention. Preventive vaccines are being developed and evaluated under laboratory and field conditions.

Many of the states, particularly those in the north central region, (NCR-47, Mastitis in Cattle) are cooperating informally in seeking to determine the cause and effective methods of controlling mastitis in cattle. Preventive and therapeutic agents are being evaluated to determine their efficacy. Residue studies are an important part of these investigations.

Attempts are being made to determine the role of certain viral agents in foot rot and infectious keratitis (pink eye).

Much effort is being made to develop means of controlling urinary calculi in cattle (Regional Research project, W-41, Urinary Calculi of Cattle). Consideration is being given to the theory that an imbalance of certain nutritional elements may contribute to the development of the condition.

Sporadic diseases and new problems not previously encountered often become economically important enough to require intensive investigation. Other bovine disease conditions currently under investigation include epizootic bovine abortion, toxicoses, ketosis, parturient paresis, white muscle disease, aplastic anemia, enterotoxemia, porphyria, tuberculosis, paratuberculosis, various abnormalities, bloat, brucellosis, Q fever, etc.

Increased attention is being paid to public health aspects of animal disease research. Greater emphasis is being placed on research which would control and eradicate animal diseases transmissible to man. The protection of the consumer against foodborne diseases is also receiving considerable support.

The total State scientific effort devoted to diseases of cattle is 132.1 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Brucellosis of Cattle

In a research study conducted at the National Animal Disease Laboratory (NADL), Ames, Iowa, fifteen bulls from 4 to 10 months old were vaccinated against brucellosis to determine the nature and frequency of persistence of Brucella abortus Strain 19. A mild transient postvaccinal orchitis was detected in 8 bulls. Postvaccinal brucellemia was detected in 13 of the 15 bulls and persisted up to 28 days in one bull. Semen samples collected from the bulls had no noticeable decrease in quality and did not contain Brucella. Postvaccinal serum agglutinin titers persisted longer at diagnostic levels in bulls than in heifers vaccinated at the same age in a previous experiment. Ten of the 15 bulls still had "reactor" or "suspect" titers at 18 months of age.

The bulls were killed at 18 months of age and at necropsy there was no evidence of gross or microscopic pathologic alterations attributable to Strain 19, nor could the organism be demonstrated in any of the tissues. Under the conditions of this experiment Strain 19 did not localize or persist in any of the 15 bulls. However, persistence of serum agglutinin titers in bulls of breeding age may confuse interpretation of test results and hinder the progress of brucellosis eradication. (NADL) (ADP al-3(R)

The University of Minnesota, under a cooperative agreement with the USDA, reported finding that the number of animals in the herd influences the efficiency and sensitivity of the Brucella Ring Test (BRT). Work is being continued on the development of improved methods for conducting the BRT to increase or decrease the sensitivity according to the number of animals in the herd. The results will greatly increase the efficiency and applicability of the BRT as a screening test for Brucella agglutinins in milk and increase the usefulness of the test in accordance with the increasing size of herds to meet economic and technologic change. (Minnesota) (ADP al-3(R)

Ohio Agricultural Experiment Station, Wooster, under a cooperative agreement with the USDA, reported progress has been made on the first and second phases of the program on serological tests and vaccination of heifer calves at 2 and 3 months of age respectively, with Brucella abortus, Strain 19 vaccine. Some of the heifers 15 to 18 months of age have been bred. (Ohio) (ADP al-3(R)

The University of Wisconsin, Madison, under a cooperative agreement with the USDA, reported finding a standardized complement fixation test, used in the diagnosis of bovine brucellosis on over 60,000 serum samples during the past three years, has been the most effective of the supplementary serological tests for the detection of infected animals regardless of the tube agglutination test titer. In no case was B. abortus isolated from an animal with a CF titer of less than + at 1:20. Over 90% of proved infected animals had CF titers of + at 1:40 or higher.

Cultures indistinguishable from Strain 19 in CO_2 independence, sensitivity to thionin blue, safranin O erythritol and penicillin have been isolated from 27 cows in Wisconsin, one from Virginia, one from Washington, one from Israel, and seven from New York over the past three years. Ten of these have also been found indistinguishable from Strain 19 in virulence for guinea pigs. These are interpreted as being authentic cases of persistent infection with Strain 19.

A method of dissociating antibody from brucella antigen in tissue by treatment with SM urea has been developed. This has been adapted to improvement of fluorescent antibody staining of Brucella abortus in tissue and to the proving of specificity of staining of antigen in a single preparation. The method should have general application in the fluorescent antibody technique.
(Wisconsin) (ADP a1-3(R)

B. Vibriosis

The New York Veterinary College, Cornell University at Ithaca, under a cooperative agreement with the USDA, reported the following:

Diagnosis of Vibriosis in the Bull by use of the Fluorescent Antibody Technique. A fluorescent antibody conjugate was prepared by labelling the gamma globulin fraction from a pool of rabbit antisera for one strain of Vibrio fetus venerealis with fluorescein isothiocyanate. Nonspecific fluorescence in stained specimens was minimized by using a fraction of the conjugate separate by ion-exchange chromotography. Cross reactions in the fluorescent antibody reaction were observed with intestinal and venereal strains of V. fetus but not with V. bubulus or 17 other species of bacteria tested.

The conjugate was used to stain smears of preputial fluid from a group of 24 bulls. This group included known carriers and bulls of varying ages from which Vibrio fetus had not been isolated. Samples from each bull were examined weekly for 6 consecutive weeks. Complete agreement was obtained between the results of the fluorescent antibody tests on preputial fluid and the results of cultural examination of semen samples from these bulls. The results of this experiment indicate that the fluorescent antibody reaction provides a highly accurate and sensitive method for the detection of V. fetus carrier bulls.

Diagnosis of Vibriosis in the Bull by Isolation of the Organism from Semen. A six-months' study has been completed for the purpose of evaluating different cultural procedures on semen for diagnosing vibriosis and obtaining an estimate of the efficiency of semen culture and as a method for detecting V. fetus carrier bulls.

Five semen samples from each of 35 Vibrio fetus carrier bulls were cultured at monthly intervals. V. fetus was identified in 151 of the 175 samples (86.3%). Of 146 isolates fully characterized biochemically all were classified as Vibrio fetus venerealis. Isolations were made in 5 of 5 attempts from 17 bulls, 4 of 5 attempts from 12 bulls, and 3 of 5 attempts from 6 bulls. On the basis of these results it would be necessary to culture two or three semen samples in order to establish a bull's carrier status with confidence. The sensitivity of this method probably falls somewhat below that of fluorescent antibody tests on preputial scrapings. Direct culturing on antibiotic medium, in which spread plates were prepared from undiluted semen from three serial tenfold dilutions, was found to be a much more efficient method of isolating Vibrio fetus than was culturing of filtered samples. (New York) (ADP al-9(R)

C. Tuberculosis

Research studies were continued at the National Animal Disease Laboratory, Ames, Iowa. The studies pertained to:

Glycoprotein Levels in Cattle Naturally and Experimentally Infected with Mycobacterium bovis. The study was to determine if serum glycoprotein changes which are used in the diagnosis of tuberculosis in other species might be applicable to the diagnosis of tuberculosis in cattle. In experimentally infected cattle there was a significant rise in the serum glycoprotein during the first five weeks after infection. After this the glycoprotein levels tended to decrease. No evidence was found correlating an increased serum glycoprotein level with either positive skin tests or the presence of lesions in naturally infected cattle. The test does not appear to be of value in the diagnosis of tuberculosis in cattle.

Concentration Effects in Cervical Tuberculin Tests of Cattle Naturally Infected with Mycobacterium paratuberculosis. In determining the effect of simultaneous intradermal cervical injections of two concentrations of ARS tuberculin on the intradermal reactions to each concentration, it was found that simultaneous injections at two concentrations did not significantly affect the size of reactions.

In determining if cattle react differently to the two concentrations, the reactions to full strength tuberculin were significantly larger than those to tuberculin diluted 1:10 whether read at 24, 48, 72, or 96 hours.

In comparing the accuracy of two methods of measuring the reactions, the interpretation of the reactions was the same whether measured by palpating or with a dermal thickness gauge.

An intravenous tuberculin test was used in 76 cattle to supplement but not replace the intradermal tuberculin test in detecting infected cattle which fail to react to the intradermal test. Forty-seven cattle reacted to the intradermal test of which 16 also showed substantial temperature increases with the intravenous test. Fourteen of the 16 had lesions of tuberculosis and 16 that did not show temperature increases also had lesions.

Twenty-nine cattle did not react to the intradermal test, 24 of these did not show temperature increases to the tuberculin administered intravenously. The remaining 5 did show temperature increases, and all 5 were found to have lesions of tuberculosis on postmortem examination.

(NADL) (ADP al-13(R)

Research was continued at the Michigan State University, East Lansing, under two contracts with the USDA. Reports submitted are as follows:

Contract No. 12-14-100-6852(45). The final report on work under this contract was received during the fiscal year 1965. Findings were:-

Polysaccharide specific antibodies and phosphatide specific antibodies, as determined by the hemagglutination (HA) test and the kaolin-phosphatide (KP) test, were elicited in calves by experimental infections with Mycobacterium bovis and M. avium. At necropsy, the calves inoculated with M. bovis had lesions and progressive disease. The calf inoculated with M. avium had lesions and non-progressive disease.

Calves inoculated with pseudochrome and Group IV atypical mycobacteria did not have increased HA and KP serum titers. At necropsy, no lesions or disease were detected.

Calves inoculated with Group III atypical mycobacteria (bovine and porcine origin) had varying HA and KP serum titers. Group III organisms (bovine origin) had a range of virulence from none to that almost equal to M. bovis and those of porcine origin produced few or no lesions in calves. The HA and KP serum titers could not be consistently correlated with disease.

The cows from a gross-lesion herd had fourfold or greater increases in HA and KP serum titers after administration of tuberculin. Two cows from a no-gross-lesion herd did not exhibit the anamnestic-like response. At necropsy the former cows had lesions and progressive disease - the latter cows did not have lesions or disease.

Swine which were inoculated with M. bovis, M. avium, and Group III organisms (porcine origin) had lesions at necropsy and fourfold increases in HA and KP serum titers after administration of tuberculin.

Three of four calves, which were inoculated intradermally with heat-killed organisms, had fourfold increases in HA serum titers. None of the four calves had lesions or disease.

The route by which some of the Group III organisms (bovine origin) were administered to cattle altered the serologic response. When inoculated intradermally, all calves had a fourfold or greater increase after the first tuberculin test.

When the three strains of Group III organisms were introduced into the uterus, four of the nine heifers did not have a fourfold increase after the first tuberculin test.

When the three strains of Group III mycobacteria were administered in an aerosol, the HA serum titers were considerably lower.

Specific phosphatide extracts prepared from a representative group of known and atypical mycobacteria did not increase the specificity of the kaolin-phosphatide test.

Generally, swine and cattle with lesions exhibited the anamnestic-like response to the first tuberculin test.

Contract No. 12-14-100-7164(45). In Section I, the study relative to virulence of 12 cultures of microbacteria in calves, swine, guinea pigs, rabbits, and chickens, is approximately two-thirds completed. In Section II the study and comparison of the disease in adult cattle and calves by Group III microbacteria, is nearing completion. In Section III progress is being made in the studies designed to produce and evaluate sensitins for the detection and differential diagnosis of microbacterial infections in laboratory animals. In Section IVa. guinea pigs were inoculated intraperitoneally with M. bovis organisms. Serums were collected from them at intervals of 7 - 10 days post-inoculation. Ouchterlony immunodiffusion was not a satisfactory way to compare serums from normal and tuberculous guinea pigs. A progressive depletion of the $alpha_1$ globulin lipoproteins and a simultaneous increase in the slow moving $alpha_2$ globulin lipoproteins occurred in the serums from the tuberculous guinea pigs.

Hyperalpha$_2$-globulinemia was detected by cellulose acetate electrophoresis in the serums from tuberculous guinea pigs. Coincident was the detection by immuno-electrophoresis of an additional $alpha_2$ globulin, tentatively named alpha$_2$-T. Cellulose acetate electrophoresis and immuno-electrophoresis of serums obtained from guinea pigs sensitized to tuberculin with heat-killed M. bovis revealed neither hyperalphaglobulinemia nor the alpha$_2$-T.

Gel infiltration of normal serum in Sephadex G-100 and G-200 was not a satisfactory procedure for the separation of the serum antigens.

Column chromatography on DEAE-cellulose separated normal serum into four major and several minor fractions.

Normal serum was separated into three main fractions by electrophoresis in insoluble potato starch. Satisfactory separation of the serum antigens was not obtained by either of these procedures. Normal serum was separated into five fractions by electrophoresis in agar gel. From seven to nine fractions were readily resolved by electrophoresis on cellulose acetate membranes. Thirty antigens were found in normal serum by immuno-electrophoresis.

Interpretation of the different phases of the work under this contract will be submitted in the final report. (Michigan) (ADP a1-13(R)

D. Mucosal-Respiratory Disease-Complex of Cattle

Research work was continued at the National Animal Disease Laboratory, Ames, Iowa. The report shows:

Studies on the intracellular synthesis, separation and characteristics of the soluble antigen of bovine viral diarrhea virus have been completed. Complement fixing soluble antigen was detectable intracellulary before the appearance of infectious virus during synthesis in roller flask cultures of bovine embryonic kidney cells. The release of infective virus into the extracellular fluid was concomitant with the release of soluble antigen.

Soluble antigen was separated from the infective virus. It was heat labile at 56 C, but stable in buffers at pH 5.0, 7.0, and 9.0 at 37C. It was irreversibly precipitated in buffers at pH 3.0 or below. Trypsin and a chymotrypsin completely inactivated the soluble antigen whereas ribonuclease and deoxyribonuclease had no detectable effect on the complement fixing activity. There was no apparent serologic relationship between the soluble antigen of bovine viral diarrhea virus and arbovirus group B and lymphocytic choriomeningitis virus antisera.

A strain of bovine viral diarrhea virus, NADL-MD, was adapted to primary and a cell line of swine kidney cell cultures.

Prior infection of the swine kidney cell line with modified hog cholera virus completely abolished the cytopathic effect and suppressed the yield of adapted NADL-MD virus. The interference occurred prior to the formation of soluble antigen since this was decreased two to fourfold and virus yield was decreased by 90 percent.

Application of the interference test made it possible to study the rate of development of neutralizing antibody against hog cholera virus.

A modified strain of hog cholera virus used to infect swine kidney cell cultures, interfered with the cytopathic effect and virus yield of adapted NADL-MD virus used for challenge. Interference by modified hog cholera virus was dosage dependent and required infection of the cells before interference was expressed. It was demonstrated that interferon prepared

by conventional methods played no role in interference. A swine-passaged strain of hog cholera virus did not produce as complete interference as the strain passaged in swine kidney cell cultures.

When cell cultures were experimentally infected with bovine virus diarrhea (BVD) and infectious bovine rhinotracheitis (IBR) viruses, the latter outgrew the former in relatively few passages, thus indicating that isolation of a mixed population of BVD and IBR viruses from an infected animal could possibly give rise to a pure culture of the latter virus in a relatively short time after the primary isolation.

Calves born to dams devoid of neutralizing antibody against bovine viral diarrhea virus (BVDV), although housed with other mature animals having antibody titers, have not become infected with the agent during a period of 8 months. When dams possessed neutralizing antibody against BVDV, it was transmitted to the calves at the same or higher titers but then decreased over the following 5 months until the serums became negative. In both of these examples the calves were kept with their dams and allowed to nurse. Results to date indicate that BVDV is not readily transmitted in the absence of clinical cases or other as yet unknown factors.
(NADL) (ADP a1-14(C)(R)

Colorado State University, Fort Collins, under a cooperative agreement with the USDA, reported that the longevity of immunity of cattle to infectious bovine rhinotracheitis (IBR) has been found to be longer than 5½ years. The quantitative relationship between neutralizing titer and the susceptibility of the cattle will give a guide for vaccination and diagnostic purposes. IBR will produce abortion. (Colorado) ADP a1-14(C)(R)

Research investigations were continued at Purdue University, Lafayette, Indiana, under a cooperative agreement with the USDA. The report shows that the apparent incidence of the mucosal disease complex has not changed. Most cases tend to be typical of chronic cases of the syndrome earlier called "virus diarrhea." However, some typical acute outbreaks of "mucosal disease-Iowa" are encountered in the Lafayette area in which mortality approaches 100% of affected animals, and morbidity 15 to 20 percent.

The properties of two cytopathogenic strains of bovine viral diarrhea (BVD) virus, Indiana 1061, and reference strain Oregon C24V, were studied using tissue culture methods. In vitro virus assays were performed in bovine kidney (BK) cell cultures. Indiana 1061 strain virus was isolated from the spleen of a calf in which pathological lesions of BVD were found at necropsy. A mild disease syndrome, indistinguishable from experimental BVD, was observed in calves that were inoculated with spleen suspension. Reciprocal cross-immunity tests demonstrated that calves immunized against Indiana 46 strain BVD virus were resistant to challenge with the new isolant. Calves that had been inoculated with spleen suspension did not react to a challenge injection of Indiana 46 strain virus.

Serum neutralization tests indicated that the newly isolated virus, designated Indiana 1061, was antigenically related to reference strain Oregon C24V. Serums obtained from calves following inoculation with Indiana 1061 and Oregon C24V viruses neutralized the cytopathic effects of Indiana 1061 virus in BK cell cultures.

Bovine kidney cell cultures infected with Indiana 1061 or Oregon C24V viruses, did not absorb guinea pig, bovine or ovine erythrocytes when incubated at 25 C. or 4 C. Erythrocytes added to the culture medium were not agglutinated.

Morphologic changes occurring in BK cells following inoculation with Oregon C24V virus showed the initial degenerative changes were confined to the cytoplasm of the cells and were characterized by condensation and vacuolization of the cytoplasm, followed by obvious nuclear changes. Cavitations in the nucleoli occurred early in the course of degeneration.

Cytochemical staining with acridine orange revealed an increase in cytoplasmic and nucleolar fluorescence (ribonucleic acid) (RNA) at 24 hours. As progressive infection of the cell sheet occurred, cytoplasmic (RNA) fluorescence increased. Treatment of cultures with RNAase completely removed the cytoplasmic and nucleolar staining.

Anti-Oregon C24V fluorescein labeled globulin specifically stained cultures infected with Indiana 1061 or Oregon C24V viruses. Specific fluorescent staining was detectable 16 hours after inoculation and prior to the development of cytopathic changes. Viral antigen was found diffusely spread throughout the cytoplasm, and in some cells was concentrated in a perinuclear location. "Rounded-up" cells showed brilliant fluorescent staining. Large particles or chunks of fluorescing material could frequently be seen in the cytoplasm of degenerating cells. In vacuolated cells, the viral antigen was concentrated at the periphery of the vacuoles. Viral antigen was not observed in the nucleus of cells. Fluorescent staining was completely or markedly inhibited when homologous immune globulin was mixed with conjugates prior to staining.

The Specific-Pathogen-Free (S.P.F.) cattle herd continues to be relatively free of important pathogens with the exception of serological evidence of parainfluenza (SF-4). Titers were highest in animals under one year but colostrum-deprived calves had no titers. The reproductive efficiency of the herd is normal and about 20 calves will be available for research during the next 12 months. (Indiana) (ADP a1-14(C)(R)

Iowa State University, Ames, under a cooperative agreement with the USDA, reported the direct fluorescent antibody (FA) test is well suited to the diagnosis of cases of infectious bovine rhinotracheitis (IBR). The method appears to be more trustworthy than isolation.

Immunological tolerance studies with bovine virus diarrhea (BVD) infected calf fetuses have been initiated with the injection of bovine fetuses in the first and second trimester of fetal life. The injection of virulent virus apparently does not cause abortion, or elicit an antibody response in the dam. Calves will be recovered by caesarean section and raised in isolation for three months prior to challenge.

Four strains of IBR are being studied in an attempt to associate basic properties with observed differences in pathogenicity. Although these strains will cross neutralize in standard neutralization tests, kinetic neutralization studies show some differences between strains.

(Iowa) (ADP a1-14(C)(R)

E. Mastitis of Cattle

The research studies at the National Animal Disease Laboratory, Ames, Iowa, related to the following:

The nonhemolytic and weakly hemolytic coagulase-negative staphylococci, known as Staphylococcus epidermidis, have been considered nonpathogenic and frequently neglected as a cause of bovine mastitis. The organisms are not typable by bacteriophage and do not produce toxins. Methods for characterizing and differentiating these organisms are needed for enzootiological studies. A study was made to determine whether the various degrees of pigmentation might be useful in differentiating strains. Spectrophotometric analysis of the pigments of 70 isolates, representing 48 strains of Staphylococcus epidermidis, exhibited absorption curves that were classified into seven types, designated I, II, III, IV, V. VI, and a S. aureus type. Two subtypes were included in types I and III. All nonpigmented cell extracts were classified as type I. Three isolates gave an absorption curve that was similar to the curve produced by extracts of five of seven S. aureus strains, thus the designation S. aureus type. The differences in pigment complexes indicated by the various absorption curves of methanol extracts were substantiated by column-chromatography studies. Generally, pigments of types II to VI, as produced by representative strains, were of a xanthophyllic nature, i.e., more soluble in methanol. The S. aureus type pigment studied was carotenelike, i.e., more soluble in petroleum ether. Analyses of representative strains showed that the type of spectral absorption curves did not change whether the organisms were carried in vitro and tested through 3 months, or isolated repeatedly from infected udders for periods up to 8 months. The method of determining the spectral absorption curves of whole-cell methanol extracts provides an additional tool for differentiating strains of S. epidermidis that can be used in enzootiological studies of udder infections.

(NADL) (ADP a1-15)

The University of California, Davis, under a cooperative agreement with the USDA, submitted a report referring to previous studies that indicated the important role of preleukocytosis (inflammatory response) in protection of the lactating bovine mammary gland against peracute coliform mastitis. Acute mastitis develops when the inflammatory reaction (leukocytes specifically) destroy the flora releasing the endotoxin. High molecular weight polysaccharides of bacterial origin have been tried as a method to prevent diapedesis of leukocytes. Commercially prepared dextran (PHARMACIA Chemicals, Uppsala, Sweden), in increasing amounts and of higher molecular weight than reported as an effective agent, gave no difference in either the speed or magnitude of the leukocytic infiltration in experimentally infected mammary glands than in glands of control cows receiving no dextran. Bacterial levans of higher molecular weight were found to be more effective than dextran in laboratory animals.

Aerobacter levanicum levan was prepared and injected into a cow prior to inoculation of a gland with Aerobacter aerogenes. The injection proved to be fatal to the cow presumably due to an overdose of the levan even though on a weight basis; less levan was injected than was the dextran. The effect resembled that of massive histamine release. No leukocytosis into the inoculated quarter took place during the 8 hours of survival post levan while the control cow developed the typical local reaction within 4 hours.

Several of the cows are now in their fifth lactation. No mammary gland infections with pathogenic staphylococci have appeared in this herd. This is evidence that staphylococcal mammary infections are not the inevitable consequence of aging and wear and tear of the milking act. The majority of these cows, although having experienced many episodes of acute or chronic experimental mastitis, secrete essentially normal, cell-free milk again within a short period of time after the pathogenic bacteria disappear.

Several natural, chronic infections with intermediate coliform organisms and paracolon-like organisms have occurred in some quarters of these cows. These infections have been persistent and have stimulated significant leukocytic infiltrations into the milk ($\pm$5.0 millions of cells/ml). Advantage has been taken of this to attempt to superimpose Streptococcus agalactiae or Pseudomonas aeruginosa in repeated daily doses without success. The pre-leukocytosis of $\pm$5.0 million cells prevented the inoculated organisms from multiplying and thus infection was precluded. Daily inoculation of Streptococcus agalactiae or Pseudomonas aeruginosa into glands having undulating levels of leukocytic infiltration have led to infection becoming established when infiltrating leukocyte level became reduced temporarily.

Pseudomonas aeruginosa appeared to be able to establish itself when multiple doses were given in the presence of infiltrating cells at $\pm$1,000,000/ml. of foremilk. Str. agalactiae even in doses of several thousand was held in check by a pre-existing inflammatory response characterized by foremilk cell counts of 500,000. Commonly, cell numbers/ml. in strippings milk are

several fold greater than in foremilk. Strippings counts of several million in the face of foremilk cell numbers of less than 1,000,000 appear to reflect a considerable barrier to both Str. agalactiae and coliform bacteria.

Cultures of fresh milk fail to demonstrate the pseudomonads. Pre-incubation of milk is required for colony growth on culture from chronically infected lactating glands. The Pseudomonas aeruginosa organisms readily grow on culture of fresh fluids drawn from chronically infected dry glands.

Studies are demonstrating the effectiveness of pre-existing leukocytosis in preventing establishment of Str. agalactiae and Ps. aeruginosa when entering the gland in small numbers as a single incident.
(California) (ADP al-15)

F. Respiratory Diseases of Cattle (Shipping Fever)

Research investigations conducted at the National Animal Disease Laboratory, Ames, Iowa, are being continued on basic studies on the physiology of organisms associated with shipping fever. A semi-defined medium for the growth of Pasteurella haemolytica was developed. It consisted of acid-hydrolyzed casein, supplementary cysteine, inorganic salts, and either D-galactose or sucrose as the carbon source. Essential vitamins were pantothenic acid, nicotinamide, and thiamine. The phosphorylated forms of thiamine were more efficient than thiamine itself in promoting growth. Six strains of P. haemolytica, isolated from cases of bovine respiratory disease, grew well in the medium. The medium is being used in studies on the effect of bovine tissue exudates and fluids upon the nutrition and metabolism of Pasteurella species.

Exposure studies in specific pathogen free calves using parainfluenza-3 virus and Pasteurella haemolytica, singly or in combination, produced a clinical syndrome closely resembling "shipping fever."
(NADL) (ADP al-17)

G. Etiology of Infertility in Cattle other than by Vibriosis and Trichomoniasis

In research studies at the National Animal Disease Laboratory, Ames, Iowa, Mycoplasma was isolated, for the first time, from an aborted bovine fetus and from vaginal mucus of cattle with signs of infertility. Morphological and biochemical comparisons were made between the fetal and vaginal strains and 4 strains from other sources. The fetal isolant differed from others in colonial morphology, methylene blue reduction and carbohydrate fermentation. Vaginal isolants were similar in colonial morphology and biochemical properties with the exception of serum requirement. Similarities were also noted between the bovine and non-bovine strains.

Additional work is needed to determine the serological relationship of the fetal and vaginal isolates as well as the importance of Mycoplasma as a cause of bovine infertility. (NADL) (ADP al-19)

H. Epizootic Bovine Abortion

The University of California, Davis, under a cooperative agreement with the USDA, reported that previous studies indicated the failure of vaccination to prevent epizootic bovine abortion (EBA) might be because the cattle were vaccinated too near the time of their exposure to the causative agent to allow for the development of a protective immunity. Multiple injections of vaccine, beginning in calfhood and continuing to or following conception, is the present program.

A viable and an inactivated vaccine are being tested in the study. The antibody response has been exceptionally good, but the immunity in these cattle has not been challenged with virulent virus as yet.

Indications are that the EBA virus is becoming attenuated by continued serial passage in chicken embryos and mice.

Preliminary findings indicate that antibiotic therapy might be of some value in controlling EBA when treatment is initiated just before or following exposure to the virus.

It was shown conclusively that the EBA agent is not spread by venereal means as once believed. (California) (ADP al-21)

I. Immunization Against Bovine Leptospirosis

Work at the National Animal Disease Laboratory, Ames, Iowa, shows that bovine leptospira were grown in chemically characterized medium free of serum or serum protein, but the leptospiral growth was poor and the organisms lacked certain surface antigens which are believed to be involved in immunity. The addition of rabbit serum or bovine albumin to cultures of L. pomona in synthetic medium did not restore their antigenicity. It was restored after a minimum of seven generations in medium supplemented with rabbit serum. Various synthetic peptides or fragments of albumin did not replace albumin. The end groups of the albumin molecule were not essential for its function. (NADL) (ADP al-25)

J. Chemotherapy in Leptospirosis

This work is being conducted at the National Animal Disease Laboratory, Ames, Iowa. The minimal, growth-inhibitory concentrations (μg./ml.) of antimicrobial agents and dyes for Leptospira pomona, Leptospira canicola, Leptospira autumnalis, and Leptospira grippotyphosa in synthetic medium were: penicillin G, 0.06; oxytetracycline and chlortetracycline, 0.5; erythromycin, 0.025; tylosin, 0.05; actinoscectacin and kanamycin, 0.25;

dihydrostreptomycin, 0.3; chelocardin and lincomycin, 2.0; fucidin, capreomycin, isoniazide, and chloramphenicol, 20.0; sulfachlorpyridazine, sulfamethoxazone, and sulfadimethoxane, 1000; crystal violet, methylene blue, and pyronin B, 0.2; and thionin and basic fuchsin, 20. Semi-synthetic penicillins (oxacillin, methicillin, and phenathicillin) were five to fifty times less effective than penicillin G. Tylosin, kanamycin, tetracyclines, and penicillins were two to five times less effective in medium supplemented with rabbit serum than in synthetic medium. No differences in sensitivity to dihydrostreptomycin were found among 12 leptospiral serotypes including virulent strains and nutritionally fastidious strains. Dihydrostreptomycin, chlortetracycline, and penicillin at high concentrations affected leptospiral respiration, motility, and viability differently. Dihydrostreptomycin had little effect on the respiration or motility of L. pomona, but subcultures failed to grow; chlortetracycline, but not penicillin, rapidly inhibited all three parameters.

Chemotherapeutic agents, which inhibited leptospiral growth in vitro, were administered to hamsters in attempts to eradicate renal L. pomona. L. pomona was cultured from only one of 50 infected hamsters treated with dihydrostreptomycin (25 mg/kg once daily for 3 days).

The following drugs (mg./kg. of body weight once daily for 3 days) were not effective: crystal violet, methylene blue, pyronin B (25); penicillin G (30 and 60); phenathicillin, oxacillin, and methacillin (25); fucidin, kanamycin, nalidixic acid, and capreomycin (25); lincomycin, chelocardin, chlortetracycline, oxytetracycline, tetracycline, chloramphenicol, actinospectacin, tylosin, and erythromycin (12.5, 25, and 50).

Neither chlortetracycline in the feed (1,000 Gm./ton for 10 days) nor dihydrostreptomycin in the drinking water (25 mg./kg. for 7 days) eliminated renal leptospires. (NADL) (ADP a1-26)

K. Investigations on Bovine Lymphosarcoma

The report from the National Animal Disease Laboratory, Ames, Iowa, shows that in the investigation of field cases of bovine lymphosarcoma, a blood protozoan parasite tentatively identified as Trypanosoma theileri, has been isolated from the leukocytes in all cases. The parasites can be detected in leukocyte cultures from 1 to 6 days after the cells have been planted. It appears essential that fetal calf serum should be used in the medium rather than serum from the affected animal in order to isolate the parasites consistently. Serum from the affected animal causes the parasite to disappear from the cultures and this may be due to antibodies. Relationship of the parasite to the cause of bovine lymphosarcoma has not been determined. (NADL) (ADP a1-30)

L. Paratuberculosis (Johne's Disease) of Cattle

Reports from the National Animal Disease Laboratory, Ames, Iowa, summarizing the work of several years, show there is no known satisfactory method of diagnosing Johne's disease in carrier animals that are not showing clinical signs of the disease.

A herd of cattle, affected by the disease, consisting of approximately 175 animals, was studied for 6 years. Blood samples were obtained from all cattle twice a year and selected tissues of all cattle slaughtered were examined. Of 159 cattle removed from the herd and slaughtered during the study, 111 had high hemagglutination test titers (1:32 or higher). At slaughter the bacillus was harbored by 45 of these of which 22 were showing clinical signs of the disease. Sixteen of the remaining 48 with low hemagglutination titers (1:16 or less), also harbored the bacillus at slaughter and 4 of these had clinical signs of disease. From these results it appears that high hemagglutination titers do not seem to be closely enough associated with active disease to use this test for diagnostic purposes on individual cattle.

Ninety-eight adult cattle from herds infected with Johne's disease were tested by injecting johnin into the jugular vein. Rectal temperatures were recorded just before the injection and at set intervals after injection. A temperature rise of 1.5° F or more over the preinjection temperature, provided the highest temperature exceeded 103.2°F, was considered a significant reaction.

All cattle were slaughtered and examined culturally and microscopically for Johne's disease. On the basis of postmortem findings, they were classified as negative, lightly infected or heavily infected. Eighty percent of the heavily infected and 35 percent of the lightly infected cattle reacted significantly to the test. The remainder did not. The intravenous johnin test appears to have value as a diagnostic test as it has better correlation with postmortem findings than any other test now in use.

(NADL) (ADP a1-35)

M. Infectious Keratitis (Pink-eye) of Cattle

At the National Animal Disease Laboratory, Ames, Iowa, in a series of preliminary experiments on bovine pink-eye, workers observed that a mercury sunlamp enhanced the effect of Moraxella bovis infection upon the bovine eye. The resulting disease was indistinguishable from field cases of infectious bovine keratoconjunctivitis (pink-eye). This method made possible the study of the disease under controlled conditions at any time of year. The workers proposed that ultraviolet has a primary etiological role in the disease. Work is being continued. Studies on the etiology of bovine pink-eye have been completed on the isolation and characterization of Moraxella bovis. (NADL) (ADP a1-37)

N. The Immunizing Effect of Brucella Cell Wall (PL 480 project)

Under a PL 480 Grant, investigations on "The Immunizing Effect of Brucella Cell Wall" are in progress at the Hebrew University, Hadassah Medical School, Jerusalem, Israel. Their report shows that killed preparations derived from Br. abortus cell walls conferred immunity to mice for up to 90 days. The period following challenge during which the mice were free from infection was very short. Animals examined at 28 days or more after challenge with high doses of bacteria were found to harbor large numbers of Brucella organisms in the spleen. Neither reinfection from extraneous sites nor the multiplication of a small number of organisms in the spleen could be implicated in the phenomenon of reversion. Vaccines prepared from cell walls of Br. abortus, melitensis and suis proved more effective than intact cells. The more recent experiments with cell wall fractions indicate that further purification of the immunizing antigen is feasible.
(Israel) (AlO-ADP-6)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Vibriosis

Dunn, H. O., Burda, Karine, Wagner, W. C., and Gilman, H. L. 1965. Isolation of Vibrio fetus from bovine semen. Cornell Vet., 55:220.

Mellick, P. W., Winter, A. J., and McEntee, K. 1965. Diagnosis of vibriosis in the bull by use of the fluorescent antibody technique. Cornell Vet., 55:280.

Mellick, P. W., Winter, A. J., and McEntee, K. 1964. Fluorescent antibody technique for diagnosing vibriosis in the bull. Proc. 5th International Cong. on Animal Reproduction and Artificial Insemination, 4:234. Trento, Italy.

Wagner, W. C., Dunn, H. O., and VanVleck, L. D. 1965. Incidence of vibriosis in an A.I. Stud. Cornell Vet., 55:209.

Tuberculosis

Larsen, Aubrey B., and Kopecky, Kenneth E. 1965. An Intravenous Tuberculin Test for Cattle. Amer. J. Vet. Res., 26:676-678.

Ray, James A., Scott, Ronald M., Niles, Clifton A., and Mallmann, Virginia H. 1963. Investigations of the Role of Heat Killed Mycobacteria and Feed Supplements of Animal Origin in Producing Tuberculin Hypersensitivity in Cattle. 67th Ann. Proc. U.S. Livestock San. Assn., October.

Mucosal-Respiratory Disease-Complex (Inf. bovine rhinotracheitis(IBR)

Chow, T. L. 1965. Bovine Mucosal Disease Complex. Auburn Vet., 21:No. 2.

Chow, T. L. 1965. Infectious Bovine Rhinotracheitis. Modern Vet. Practice, 46:52-53.

Gutekunst, D. E. 1964. Characterization of a Bovine Viral Diarrhea Agent. M.S. Thesis, Iowa State University, Ames.

Gutekunst, D. E., and Malmquist, W. A. 1965. Soluble Antigen of Bovine Viral Diarrhea Virus: 1. Intracellular Synthesis, Separation, and Characterization. Arch. Gesamte Virusforsch, 15:159-168.

Owen, N. V., Chow, T. L., and Molello, J. A. 1964. Bovine fetal lesion experimentally produced by infectious bovine rhinotracheitis virus. Amer. J. Vet. Res., 25:1617.

Pirtle, E. C., and Gutekunst, D. E. 1964. Relationship between the Soluble Antigens of Hog Cholera and Bovine Viral Diarrhea Viruses Prepared in Tissue Culture. Vet. Record, 76:1177.

Bovine Virus Diarrhea

Ramsey, F. K. 1963. The Pathology of Bovine Mucosal Disease-Virus Diarrhea Complex. Proc. XVIIth World Vet. Cong., Hannover, Germany, August 14-21.

Ramsey, F. K., and Tyler, D.E. 1964. The Bovine Viral Diarrhea-Mucosal Disease Complex. Iowa Vet., 35, No. 3. May-June.

Mastitis

Sandvik, Olav, and Brown, Richard W. 1965. Spectrophotometric characterization of pigments produced by Staphylococcus epidermidis strains isolated from bovine udders. J. Bact., 89:1201.

Respiratory Disease of Cattle (Shipping Fever)

Marshall, R. G. 1944. Isolation of Bovine Parainfluenza-3 virus in chick embryos. J. Bacteriol. 88:267-268.

Wessman, G. E. 1964. Interrelationships of Smooth and Nonsmooth Variants in the Dissociation of Pasteurella haemolytica. J. Bacteriol. 88:356-60.

Wessman, G. E. 1965. Cultivation of Pasteurella haemolytica in a Casein Hydrolystate Medium. Appl. Microbiol. 13:426-431.

Epizootic Bovine Abortion

McKercher, D. G. 1964. Some viruses associated with the respiratory tract of cattle and the syndromes which they cause. Nord. Vet. Med., Suppl. 1, 16.

McKercher, D. G., McGowan, B., Wada, E. M., Harrold, D. R., and Studdert, M. J. 1964. Isolation of the virus of enzootic abortion of ewes from California and Oregon sheep. Jour. AVMA, 145:564-569.

Studdert, M. J., Wada, E. M., Kortum, W. M., and Groverman, F. A. 1964. Bovine infectious pustular vulvovaginitis in the western United States. Jour. AVMA, 144:615-619. Supported by USDA Grant 12-14-100-5297(45)

Immunization against Bovine Leptospirosis

Stalheim, O. H. V. 1965. Nutritional Aspects of Leptospiral Antigenicity. Bacteriol. Proc., p. 54.

Chemotherapy in Leptospirosis

Stalheim, O. H. V. 1965. Leptospiral Lysis by Lipids of Renal Tissue and Milk. J. Bacteriol., 89:545.

Bovine Lymphosarcoma

Malmquist, W. A. 1965. Trypanosomes in Leucocyte Cultures. Vet. Record, 77:350.

Paratuberculosis of Cattle (Johne's Disease)

Larsen, A. B., and Kopecky, K. E. 1965. Studies on the Intravenous Administration of Johnin to Diagnose Johne's Disease. Amer. J. Vet. Res., 26:673-675.

Larsen, A. B., Vardaman, T. H., Kopecky, K. E., and Merkal, R. S. 1965. An Extended Study of a Herd of Cattle Naturally Infected with Johne's Disease: The Significance of the Hemagglutination Test. Amer. J. Vet. Res., 26:254-257.

AREA NO. 2 - INFECTIOUS AND NON-INFECTIOUS DISEASES OF SWINE

Problem. Profitable swine production depends largely on the ability to control diseases. Swine diseases cause losses estimated at more than $200 million annually. In order to control and eventually eradicate these diseases, a thorough knowledge of causes, diagnostic procedures, preventive procedures, and treatments is required. Although a great deal of excellent research has been and is being accomplished, a vast amount of research is still required to obtain this knowledge. At present, the causes of several important swine diseases are unknown or incompletely understood. Extensive fundamental research on swine diseases is essential to the welfare of the swine industry.

USDA AND COOPERATIVE PROGRAM

The Department has a long history of swine disease research. For example, research on hog cholera was initiated in 1884. Research on this and other important swine diseases is a continuing long-term program. Modern research techniques in the areas of biochemistry, biophysics, pathology, microbiology, pharmacology, physiology, and immunology, are being applied to swine disease problems. Research is being conducted on the following diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 14.2 professional man-years. This effort is divided among sub-headings as follows:

Hog Cholera 6.4 at the National Animal Disease Laboratory, Ames, Iowa, the Florida Hog Cholera Research Station, Live Oak, Florida, under a cooperative agreement with the University of Illinois, and under a contract with the University of Nebraska.

Atrophic Rhinitis 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

Transmissible Gastroenteritis 2.3 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with Purdue University and the University of California.

Erysipelas 1.5 at the National Animal Disease Laboratory, Ames, Iowa.

Brucellosis 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Abscesses 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

PROGRAM OF STATE EXPERIMENT STATIONS

Swine disease research at the State stations involves a continuing program of investigations to bring about improved means of control for the major problems present in this country. A considerable amount of this effort involves basic research aimed at providing new information applicable to a more complete understanding of these problems.

Swine enteritis is receiving particular attention through a coordinated attack by means of the regional research program (NC-62). Ten States and the Department are cooperating on various phases of this problem.

Respiratory diseases such as atrophic rhinitis, virus pig pneumonia and influenza are under study at several locations. The Specific Pathogen-Free system of controlling these conditions is undergoing evaluation for further improving this disease control method. Germ-free pigs are being used by several stations in studying enteric and respiratory diseases of swine.

Investigations are continuing in order to find improved methods for diagnosis and vaccination against hog cholera. Renewed efforts are being made to develop procedures which may be found practical in controlling jowl abscesses of swine.

The role of sensitization phenomena in causing the arthritic form of erysipelas is under study. The cause of gastric ulcers, and means of preventing them, are being determined. The toxicity of certain agricultural chemicals for swine is under investigation.

The total State scientific effort devoted to swine research is 42.0 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Hog Cholera

Research at the National Animal Disease Laboratory, Ames, Iowa, was conducted in the following phases:

1. Inactivation of Hog Cholera Virus in Blood and Excreta with Chemical Disinfectants. Of 13 chemical agents tested, only one chemical, beta-propiolactone,0.6%, inactivated the virus in defibrinated blood with a titer of 7.5×10^5/ ml. Wheaton's Safety Disinfectant, 1%, was the only one of the other 12 chemicals which killed the virus in blood diluted 50%. Roccal, 2%; cresol, 2%; sodium hydroxide, 2%, and sodium hypochlorite, 1%, killed the virus in 10% blood. The other 8 compounds tested, Nolvasan, Clorox, septisol, sodium carbonate, ethyl alcohol, formaldehyde, phenol, and isopropyl, were either only partially effective or had no effect on the virulence of the virus.

Beta-propiolactone, 0.15%; Wheaton's Safety Disinfectant, 0.5%; Roccal, 2%; cresol, 2%, and sodium hydroxide, 2%, killed hog cholera virus in fecal suspensions. Sodium hydroxide, 3%, did not kill hog cholera virus in defibrinated blood.

Except for sodium hydroxide, none of the effective agents gave extreme pH values. Three of the inactivating agents were effective in diluted blood at pH values near neutrality.

2. Diagnosis of Hog Cholera. A field evaluation study, started in 1963, of the fluorescent antibody-tissue culture test (FATCT) for the routine diagnosis of hog cholera (HC) has been completed. FATCT was found to be an effective method for HC diagnosis. However, information gained from this study indicates that the inoculation of HC susceptible swine with suspect material is a more sensitive diagnostic procedure than FATCT. In all instances the identification of HC virus by FATCT was subsequently confirmed by animal inoculation (228 outbreaks), whereas a diagnosis of HC was made by animal inoculation but not FATCT with tissue samples from 8 suspected outbreaks of HC. The final correlation between the two tests was 96.6 percent.

It was suspected that tissue culture cells were less susceptible than swine to infection with HC virus. To test this hypothesis, a comparative titration was performed employing the Ames strain of HC virus. The same dilutions of virus were injected into specific-pathogen-free (SPF) swine and onto cells of the PK-15 swine kidney cell line. Titers were $10^{6.64}$ and $10^{7.17}$ for tissue culture and swine, respectively. The results would thus tend to support the hypothesis. However, it must be emphasized that only one comparative titration was performed employing a single strain of HC virus.

Spleen tissue was superior to blood for conducting FATCT. Hog cholera virus was isolated and identified from 91% of all spleens received from confirmed cases of HC, whereas HC virus was isolated from only 60% of the blood samples.

Since HC virus was not identified by FATCT from a significant percentage of spleens (9%) submitted from swine herds where HC was known to exist, it was concluded that multiple sampling is of paramount importance, that is, two or more spleens should be submitted from any suspected outbreak.

(Ames, Iowa. NADL) (ADP a2-17)

In research being carried out under a cooperative agreement at the University of Illinois, Urbana, the basic observation was made on the effect of high oxygen concentration on cytopathogenicity of hog cholera virus in tissue cultures. Briefly, it was found that rabbit testicular cell cultures inoculated with spleen extracts or blood derived from pigs infected with hog cholera virus showed marked cellular destruction after incubation at 37 C in an atmosphere of 95% oxygen and 5% carbon dioxide, at a pressure of 5, 10, or 15 pounds per square inch. No cellular destruction was observed in

cultures similarly inoculated, but incubated at normal atmospheric pressure, nor in uninoculated cultures that were incubated under increased O_2-CO_2 pressure. That the cytopathic effect (CPE) observed in cultures inoculated with hog cholera virus was indeed caused by the virus was confirmed by the finding that CPE was prevented by hog cholera antiserum but not by normal swine serum. However, it was not possible to reproduce the CPE for more than a few serial passages utilizing the fluid of tissue cultures in which CPE had been induced by hog cholera virus. This observation suggested that the CPE was caused by a cytotoxin either associated with the virus or produced by the virus-infected cells, and that the expression of CPE was not accompanied by viral replication. (Urbana, Illinois)

In research conducted under contract at the University of Nebraska, Lincoln, a highly promising test for the diagnosis of hog cholera using a fluorescent antibody staining technique has been developed. The test is accomplished directly on fresh tissues such as tonsil, lymph nodes, salivary gland, and kidney. In experimental cases, hog cholera virus could be detected in the tonsil as early as 72 hours after exposure. (Lincoln, Nebraska)

3. Chromosomal variations in a pig kidney cell line persistently infected with hog cholera virus. In work at the National Animal Disease Laboratory, Ames, the number of chromosomes occurring most frequently (modal number) has been determined for two pig kidney tissue culture cell lines (both designated PK-15) from two different sources. The majority of the cultured cells of the PK-15(NADL) cell line contained 38 chromosomes, the same number found in the living pig. In contrast, the majority of cultured cells of the PK-15(ATCC) cell line contained 37 chromosomes, one less than typical for the living pig. Both of the PK-15 cell lines contained a large, unmatched, identifying chromosome.

A pig kidney cell line, with cells, most of which contained 38 chromosomes, was infected with virulent hog cholera virus and subcultured 84 times. The continued presence of hog cholera virus during subculturing was confirmed by a specific staining test. Chromosome studies were made on cells prepared at the 82nd subculture, and the findings compared with noninfected cells subcultured in a similar fashion. It was found that most of the infected cells contained 37 chromosomes. In those cells containing 37 chromosomes some of the groups were observed to have lost one or more chromosomes and this was accompanied by the simultaneous appearance of new unpaired chromosomes in other groups. Even though there were numerous chromosomal rearrangements in the infected cells, a large identifying "marker" chromosome present in the noninfected cells was also observed in the infected cells. (NADL)

4. Soluble antigens of hog cholera and bovine viral diarrhea viruses. Partially purified soluble antigens of hog cholera -- and bovine viral diarrhea -- infected tissue cultures were prepared by freeing crude preparations of most of the infective virus.

When the hog cholera and bovine viral diarrhea soluble antigens were tested in agar-gel plates with either hog cholera or bovine viral diarrhea antiserum, a continuous line of precipitation was obtained. The specificity of the reactions was confirmed by the fact that the test antigens did not react with nonimmune sera and the antisera did not react with control antigens prepared from noninfected control antigens. (NADL) (ADP a2-17(C)

5. Pilot field studies to evaluate diagnostic tests, biologic products, and quarantine measures for a hog cholera eradication program. Preliminary exploratory studies were conducted on reactions of pigs to sublethal doses of virulent hog cholera virus. The object of these studies was to establish a foundation of experimental evidence for further research on the immunizing effect of repeated, increasingly larger but sublethal doses of virulent hog cholera virus on swine, to demonstrate the variations in reactions in different swine associated with exposure or challenge with minimal infecting doses of virus and to present other uses of minimal infecting doses of live virus in swine such as for virus characterization.

Sixty-two pigs and two strains of virus were used in these studies. Thirty-one pigs died with symptoms and lesions at necropsy usually associated with hog cholera following the first exposure to hog cholera virus. These pigs were considered to have been non-immune (susceptible). The other 31 pigs survived the initial exposure to hog cholera virus and were given increasingly larger but sublethal doses of virus. Twenty-one of these also survived one or more subsequent exposure to hog cholera virus but did not develop complete immunity. Upon exposure to greater doses of virus, they developed hog cholera and died. These pigs were considered to have been partially immune. Five of these 21 pigs, all of which had been treated with the same strain of virus, had hog cholera reactions following doses of diluted virus and recovered. Upon challenge with larger doses of virus, however, they were found not to have developed complete immunity. In other words, the hog cholera reaction in these five pigs did not indicate an immune response and may have indicated a characteristic of this particular strain of virus.

Ten pigs survived the initial exposure and subsequent exposures to diluted but increasingly larger doses of virus and developed complete immunity. This was demonstrated by their survival following subsequent challenge exposure to 1.0 ml. of undiluted, virulent hog cholera virus.

Hog cholera virus in order to be pathogenic for swine seemed to require a quantitative minimum (particulate) as well as a qualitative minimum (virulence) of virus.

The pilot hog cholera (HC) eradication project in Lowndes County, Georgia, was initiated December 29, 1961, and was terminated 30 months later on July 31, 1964. The objectives of the study were to test the efficacy of killed HC virus vaccines for eliminating HC virus from an area of known infection and to develop methods of identifying and preventing virus

reintroduction and dispersion following virus elimination. The vaccination program consisted of 3 periods - 1) initial government-paid vaccination of all swine in the county within a 2-month period; 2) government-paid vaccination of swine increases for 25 months and starting with the 2nd month of initial vaccination, and 3) owner-paid vaccination for 4 months.

The results of the field trial study demonstrated that a high population protection level (83.7%) against hog cholera was initially obtained during the first period with 93.0% vaccination coverage of all swine multiplied by the 90.0% immunity obtained at that time, with two 5 ml. doses of killed-virus vaccine. This procedure rapidly eliminated clinical hog cholera from the test area. However, evidence of nonclinical hog cholera, measured by the resistance to challenge with virulent HC virus of nonvaccinated controls left in vaccinated herds, continued to persist in 4 of 29 herds tested during 6 months of the second vaccination period. There were 41 of 41 non-vaccinated controls, each from different herds, which showed no resistance to HC virus challenge during the next 8 months, which indicated clinical and nonclinical HC virus had been eliminated. During this time, the swine population protection level against HC dropped from 83.7% to 40.0% because of a decline in swine vaccinations that continued throughout the rest of the project period, while immunity levels remained relatively constant.

During the last 11 months of the second vaccination period, as the population protection level dropped from 40.0% to 18.0%, and the third vaccination period of 4 months when the population protection level dropped from 18.0% to 10.0%, 6 of 53 vaccinated herds were positive for nonclinical hog cholera. This incidence suggested sources of HC virus introduction into the area.

The simultaneous vaccination of market swine, with killed vaccine and anti-HC serum, before movement to test area farms was an effective method of controlling the dispersion of HC virus from markets to Lowndes County farms. No clinical or nonclinical positive HC was identified in herds receiving purchased swine even though 42 outbreaks of HC occurred in adjoining counties during the test period.

The average immunity protection of 2007 killed HC virus vaccinated swine challenged with virulent HC virus, was 77.1 percent. Double vaccination with killed-virus vaccines produced better immunity protection (78.7%) than did double vaccination with killed-virus vaccines and anti-HC serum (73.6%) or single vaccination (68.8%). The duration of immunity from double vaccination at 10 months postvaccination (76.9%) was superior to single vaccination (56.6%). Double vaccination of weanlings gave better immunity protection (84.6%) than the double vaccination of suckling-weanlings (69.1%) or sucklings (69.6%) as compared with the single vaccination immunity protection of weanlings (70.1%) and sucklings (58.7%).

(Live Oak, Florida) (ADP a2-13)

B. Atrophic Rhinitis

At the National Animal Disease Laboratory, Ames, Iowa, research on atrophic rhinitis (AR) of swine was reinitiated during the year. The work of the laboratory personnel was applied to solving basic problems of raising specific-pathogen-free hysterectomy-derived pigs, developing tissue culture cell lines, and developing isolation and identification procedures for bacteria. These various techniques and methods will be used as tools to identify the causes of AR. (NADL)(ADP a2-8)

C. Transmissible Gastroenteritis (TGE)

At the National Animal Disease Laboratory, Ames, Iowa, five isolates of transmissible gastroenteritis (TGE) from different geographical areas have been studied and compared. All isolates studied have a common cytopathic virus which has biological properties which indicate that it belongs to the myxovirus class. Concurrent research indicates that this cytopathic virus is not the most important virus involved in TGE, but it appears to be present in many of the outbreaks of TGE which have been studied, and most TGE convalescent serum samples from outbreaks in the field contain antibodies which will neutralize the cytopathic virus.

Evidence of the cytopathic virus can be removed by the use of specific homologous antiserum. Cell cultures so treated remain pathogenic for pigs, however. When the cytopathic virus is purified by plaque picking techniques it is also pathogenic for pigs. Either virus alone seems to produce a somewhat different disease in susceptible pigs than is expected from feeding virus-bearing intestinal tissue or primary swine kidney cultures containing both viruses. (NADL) (ADP a2-10)

At Purdue University, Lafeyette, Indiana, under a cooperative agreement, further investigation into the pathogenesis of transmissible gastroenteritis (TGE) showed that this virus caused a severe and rapid atrophy of the intestinal villi of pigs. The villi are hair-like projections lining the entire small intestine and are essential to the digestion and absorption of nutrients. The extent of the change was observed by using an instrument designed at this laboratory which permits examining the entire intestine under a dissecting microscope in a relatively short time. The lesions were also examined by measurement of villi and characterization of the cellular changes in microscopic sections of pigs killed at all stages of the disease. The results of this work showed that villi were atrophied throughout the small intestine except for the first few inches without observable inflammatory reaction within 24 to 48 hours after inoculation of virus. The cells covering the remaining stubs of villi were not differentiated into the normal columnar cells. Villus atrophy was correlated with decreased ability to absorb orally administered glucose. The possibility that this change was the result of inhibition of cell multiplication by the virus was eliminated by counts of mitotic figures after administration of colchicine to infected pigs. Mitotic activity in the intestinal mucosa was increased in these pigs.

Villi showed beginning re-growth in pigs killed as early as 5 days after infection and apparently returned to functional normalcy through the next few days. This was correlated with cessation of diarrhea.

The results of this work would characterize TGE as an acute malabsorptive disease caused by a virus. This information will contribute to the diagnosis and ultimate development of treatment of TGE and, additionally, is a contribution to the understanding of diarrheal disease in general.

Work on immunity extended previously reported work on the mechanism of transfer of passive immunity to TGE from sows to pigs. It was shown that pigs which had suckled immune sows and, therefore, had absorbed antibody from these sows were as susceptible, after removal from those sows, to minimal doses of virus as pigs which had suckled non-immune sows. Pigs left to suckle immune sows were resistant to 1000 times as much virus as that which infected those removed from them. This passive immunity then, must result from inactivation of virus by antibody in milk within the lumen of the alimentary tract. This, as far as is known, is the first proof of this type of transfer of passive immunity. The term "lactogenic immunity" is used to describe it. (Lafayette, Indiana)

In work at the School of Veterinary Medicine, University of California, Davis, under a cooperative agreement with the USDA, the Chico strain of enterovirus was isolated from pigs in a herd showing signs of vomiting and diarrhea with a negligible mortality.

No symptoms could be demonstrated in naturally reared pigs inoculated by any route under normal experimental conditions but when inoculated pigs were exposed to 15 or 30% CO_2 in air following intravenous and oral inoculation, signs of vomiting and diarrhea were reproduced.

Specific-pathogen-free (SPF) pigs inoculated with the Chico virus intravenously and exposed to a CO_2 atmosphere, resulted in a nervous disorder as well as vomiting and diarrhea. Previous trials in SPF pigs in the absence of stress did not result in any visible signs following any route other than the intracerebral route.

Expose of pigs to CO_2 atmosphere following infection with strain El enterovirus did not influence the course of the disease syndrome which was caused by the virus in the absence of stress. (Davis, California) (ADP a2-10)

D. Erysipelas

At the National Animal Disease Laboratory, Ames, Iowa, a summarization of "Physiopathological Studies of Erysipelas in Pigs" was submitted for the year 1964, and the article published in January of 1965.

Serotypes A, B, and N of Erysipelothrix rhusiopathiae have been isolated from a variety of domestic animals and fish and in turn have been related to clinical aspects of swine erysipelas and animal species. Thus, the use

of serotyping could increase the knowledge of the epizootiology of swine erysipelas if the organism retained its particular serotype after passing through the host. Some published reports indicated that a change in serotypes occurred after repeated transfers in liquid medium and after numerous passages in mice. An assumption was made that this could occur in pigs. From the results of a study at this laboratory, it was concluded that 1) no change occurred in the serotype of strains after passage through either susceptible pigs or ones that had been immunized with either killed cells of a homologous or heterologous type, and 2) no change occurred in the serotype after storage on solid medium at room temperature for approximately 3 months.

Arthritis in pigs can be a sequela of acute swine erysipelas and constitutes a sign of the chronic form of the disease. Some researchers believed that arthritis also can appear in healthy farm pigs with no history of swine erysipelas and suggest that arthritis is an independent form of the disease. Others believed that arthritis can be the result of hypersensitization to Erysipelothrix rhusiopathiae. The latter aspect has received considerable attention because of implications with human arthritis, but experimental evidence to support the relationship between arthritis and hypersensitization in pigs has not been conclusive. An objective of one study was to determine in specific-pathogen-free pigs if the arthritis associated with erysipelas is caused primarily by local infection or by sensitization. From the results of this series of experiments it was concluded that 1) the arthritis of swine erysipelas is caused by specific infection rather than hypersensitivity; 2) there was no significant relationship between the serotype of the challenge and the incidence of arthritis in immunized pigs; 3) the degree of virulence was not necessarily associated with the ability of an organism to induce arthritis in immunized pigs, and 4) arthritis occurring in immunized pigs supports the field observations that arthritis is seen in pigs with no apparent history of swine erysipelas.

Failure to recover the organism from some joints has been explained as due possibly to 1) its elimination by natural processes; 2) localization in an area not reached by the swab, and 3) localization in an area not opened for examination. The significance of items 2 and 3 was demonstrated when the known, but heretofore overlooked anatomical structure of the carpus and tarsus was reviewed. The carpus has 3 synovial sacs and the tarsus has 4, and all do not communicate, so that infection in one sac would not necessarily involve the other sacs. Thus, when histologic preparations are made, a sagittal section through the entire articulation should include all the synovial sacs and the bacteriologic examination should include material from more than one synovial sac. (NADL) (ADP a2-15)

E. Brucellosis

At the National Animal Disease Laboratory, Ames, Iowa, the following work is reported.

Since the pathogenesis of swine brucellosis has not been thoroughly investigated, an experiment designed to gain more information about that aspect of the disease was conducted. Seven sexually mature boars were exposed to a representative strain of Brucella suis, type 1, and seven to a representative strain of Br. suis, type 3. One boar from each group was killed at each of the following postexposure intervals - 1, 2, 3, 4, 6, 8, and 12 weeks.

Severe clinical signs of the disease occurred in only a few boars and these signs were attributed to pathologic changes occurring in accessory genital glands.

Agglutinins appeared in detectable amounts in the serum of boars about one week postexposure, reached their maximum at two weeks, then gradually receded thereafter. The presence of Brucella agglutinins was also demonstrated in secretions or exudates from seminal vesicles bearing gross lesions. All boars experienced a period of sustained brucellemia after exposure.

At necropsy, 75% of the Br. suis isolations were made from lymph nodes, 15% from the urogenital system, and 10% from other tissues. Gross pathologic changes attributable to both types of Br. suis were primarily confined to seminal vesicles and their regional lymph nodes. Histopathologic alterations were most frequently observed in lymph nodes, liver, accessory genital glands, and bones of infected boars. All anatomic systems were represented in the bacteriologic and pathologic examinations.

The period of maximum infection extended from 2 through 6 weeks postexposure with 92% of the Br. suis isolations and 82% of the histopathologic alterations occurring in boars killed during that period. The disease produced by Br. suis in boars could be classified as a subacute to chronic, proliferative disease affecting primarily the reticuloendothelial system and accessory genital glands.

Since brucellosis is often a venereal disease in swine, one of the most important reservoirs of the disease is breeding herds. Considerable information has been gained through conducting this experiment on the pathogenesis of brucellosis in boars. A similar study should be conducted with female swine, with particular emphasis on changes in the genital tracts of pregnant females infected with Br. suis. (NADL) (ADP a2-16)

F. Abscesses

Research on abscesses in swine has been under way at the National Animal Disease Laboratory at Ames, Iowa, during the past year but the results have not progressed to the reporting stage. (NADL) (ADP a2-19)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Hog Cholera

Beall, C. W., Zinober, M. R., Mott, L. O., Kemeny, L., and Stewart, W. C. 1965. Hog Cholera Eradication Program in Lowndes County, Georgia, Using Killed-Virus Vaccines: Final Progress Report. Proc. 68th Ann. Meet. U. S. Livestock Sanit. Assn., pp. 284a - 284r.

Mengeling, W. L., and Torrey, J. P. 1965. The Diagnosis of Hog Cholera by the Fluorescent Antibody Technique. Paper given at FAO/OIE International Meet. on Hog Cholera and African Swine Fever, Rome Italy, May 31-June 5.

Pirtle, E. C., and Gutekunst, D. E. 1965. Relationship between the soluble antigens of hog cholera and bovine viral diarrhea viruses prepared in tissue culture. Vet. Rec., 76:1177-1178.

Pirtle, E. C. 1965. A soluble precipitating antigen (HCA) from hog cholera virus propagated in tissue culture. II. Incidence of HCA-antibodies in sera of hog cholera-immune and nonimmune swine. Canad. J. Comp. Med. and Vet. Sci., 29:90-93.

Pirtle, E. C., Rebers, P. A., and Weigel, W. W. 1965. Nitrogen-containing and carbohydrate-containing antigen from Actinomyces bovis. J. Bacteriol., 89:880-888.

Torrey, J. P., and Amtower, W. C. 1965. Inactivation of hog cholera virus in blood and excreta with chemical disinfectants. Proc. 68th Ann. Meet. U. S. Livestock Sanit. Assn., pp. 287-298.

Transmissible Gastroenteritis

Hooper, B. E. 1965. Transmissible gastroenteritis of swine: Studies on the pathogenesis. Ph.D. Thesis, Purdue University.

McClurkin, A. W. 1965. Studies on Transmissible Gastroenteritis of Swine. I. The Isolation and Identification of a Cytopathogenic Virus of Transmissible Gastroenteritis in Primary Swine Kidney Cell Cultures. Canad. J. Comp. Med. and Vet. Sci., 29:46-53.

Yamanouchi, K., Bankowski, R. A., and Howarth, J. A. 1964. Pathogenicity of E1 and E4 strains of Porcine Enteroviruses in specific pathogen free pigs. J. of Infec. Dis., 114:450-457.

Erysipelas

Shuman, R. D., Wood, R. L., and Nord, N. 1965. The Effect of Passage in Pigs on Erysipelothrix rhusiopathiae with Reference to Serotype. Cornell Vet., 55:233-242.

Brucellosis

Deyoe, B. L. 1965. The Pathogenesis of Brucellosis in Male Swine. M.S. Thesis, Iowa State University Library, Ames, Iowa.

AREA NO. 3 - INFECTIOUS AND NON-INFECTIOUS DISEASES OF SHEEP AND GOATS

Problem. There are at least 18 infectious diseases of sheep and goats in the United States that cause an estimated annual loss of 15 million dollars. Non-infectious diseases are estimated to cause an additional 3 million dollar loss annually. The cause of some of these diseases is known; others have more than one causative agent contributing to produce the effects seen in field cases. Environmental, genetic, and unknown factors appear to play a part in some diseases. The natural reservoirs of the known infectious agents have not been fully determined. Fundamental information on methods of transmission and means of prevention are needed for many of these diseases. Vaccines and other immunizing products are available for some diseases of sheep but not for others. Some of these products might be improved. Prevention, control, or eradication of disease is necessary for economic and efficient sheep and goat raising. Due to lack of accurate, rapid diagnostic techniques, infectious diseases often get a substantial start in a band or flock before they are recognized, partly because they are easily confused with non-infectious diseases.

USDA and COOPERATIVE PROGRAM

The Department has a continuous long-term program involving biochemists, microbiologists, pathologists, and veterinarians engaged in both basic studies and the application of known principles to the solution of infectious and non-infectious diseases of sheep and goats. Research is being conducted on the diseases at the following designated locations.

The Federal scientific effort devoted to research in this area totals 7.5 professional man-years. This effort is applied as follows:

Bluetongue 4.0 at the Animal Disease Research Laboratory, Denver, Colorado.

Vibriosis 0.2 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the Colorado, Montana, and Utah Agricultural Experiment Stations.

Scrapie 0.2 at the Agricultural Research Council Field Station, Compton, Berkshire, England, and the Moredun Institute, Edinburgh, Scotland, through two grants of PL 480 funds. The work is coordinated through the European Mission for Research on Animal Diseases, Amsterdam, Holland.

Paratuberculosis 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Ulcerative dermatosis 0.1 under a cooperative agreement with the Colorado Agricultural Experiment Station, Fort Collins.

Toxicological Effects of Oxalate-Containing Plants 1.0 at the Poisonous Plants Research Laboratory, Logan, Utah.

Identification of Teratogenic Agent in Veratrum californicum 0.5 at the Poisonous Plants Research Laboratory, Logan, Utah.

Chronic Toxicity of Herbicide Accumulation in Sheep Tissues 0.5 at the Poisonous Plants Research Laboratory, Logan, Utah.

PROGRAM OF STATE EXPERIMENT STATIONS

Increasing application is being made of basic scientific disciplines such as biochemistry, physiology, endocrinology, and virology in determining the causes and methods for control of diseases of sheep and goats.

States in the West (Regional Research project, W-27, Vibriosis in Sheep) are continuing their efforts to develop methods for preventing and controlling vibriosis. Considerable progress is being made in developing effective vaccines and therapeutic materials. Information is being sought on the pathogenicity and antigenic relationships of the various strains of vibrio organisms.

Basic studies (Regional Research project W-41, Urinary Calculi in Cattle and Sheep), pertaining to the influence of nutrition on the physical and chemical properties of urine seek to determine the cause of urinary calculi. Methods for prevention and treatment are being evaluated.

There is continuing interest in the prevention and control of white muscle disease (myodegeneration) in sheep. Attempts are being made to relate the findings to similar conditions in other animals, including man.

Vaccines for the prevention of bluetongue are being evaluated in several states. Vectors, in addition to those already known, are being studied in order to improve present control measures.

Epididymitis, arthritis, and ulcerative dermatosis have become economic problems in some areas and workers in several states are devoting considerable effort to determining possible means of control.

Sheep and goats are being used increasingly in studies on toxicoses, ketosis, bloat, pneumonia, and hyaline membrane disease. Such studies are designed to provide basic information which may be related to similar conditions in other animals, including man.

Other sheep and goat diseases being investigated by workers in various states are - listeriosis, ovine virus abortion, scrapie, enterotoxemia, etc.

The total State scientific effort devoted to diseases of sheep and goats is 19.9 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Bluetongue

At the Division's Animal Disease Research Laboratory at Denver, Colorado, the following work was reported:

The Transmission of Bluetongue Virus to Embryonating Chicken Eggs by Intrathoracically Infected Culicoides variipennis. Experiments in which a 100 percent infection rate of Culicoides varripennis was achieved by intrathoracic inoculation of the virus, demonstrated that a 100 percent transmission rate to embryonating chicken eggs was accomplished with flies that were incubated from 6 - 28 days after inoculation and that took a definite blood meal in feeding on the recipient host. The transmission rate was also 100 percent for all younger flies incubated from 6 - 15 days, regardless of the type of meal taken. Three transmissions with flies incubated 3 to 6 days after inoculation with virus indicated the probe of a fly was sufficient in itself to transmit the disease. These experiments showed that 100 percent of the flies were able to transmit the disease in from 3 - 5 days after inoculation of the virus into the insect's hemacoel. C. varripennis is thus demonstrated to possess qualities that are apt to make it an excellent vector in the field, at least insofar as bluetongue disease is concerned.

Replication of Bluetongue Virus in the Salivary Gland Cells of Culicoides variipennis. Bluetongue virus has been discovered and photographed in the cells of the salivary gland of Culicoides variipennis, a known vector of bluetongue diseases. C. variipennis (gnat) has been infected with bluetongue virus and the salivary glands were dissected free of the insect and studies made in the electron microscope. The method of replication of the virus in the salivary glands cells is being established. This basic piece of research is the first successful ultracytopathological study of an arbovirus in insect cells.

Cytopathology of Bluetongue Virus in Cultured Cells. Bluetongue viral antigen can be detected by fluorescent antibody techniques around the plasma membrane at 20-30 hours after viral inoculation of the culture. The intensity of the fluorescence moves toward the nucleus and becomes most intense around the nucleus at 40-50 hours after inoculation. The specific fluorescense congregates again around the plasma membrane just before the cell undergoes lysis.

Inclusion bodies are seen to form in the cytoplasma near the nucleus at 28-36 hours after viral inoculation of the culture. These inclusion bodies do not contain specific viral antigen when first formed. The fluorescense is first seen as a halo around the inclusion body and progressively invades the inclusion until the entire structure fluoresces brightly. The inclusion bodies are believed to be either lysosomes and/or buddings from the nucleus.

Multiplication of Bluetongue Virus in Culicoides variipennis following Artificial Inoculation. Bluetongue virus was proven to have multiplied in Culicoides variipennis following the intrathoracic inoculation of the virus into the insect. Virus increased as much as 1,000 to 10,000-fold during the first 7 days following infection of the insect and remained at a high level in the insect for over 3 weeks. Experiments were conducted to determine the influence of the amount of virus inoculated and the duration of incubation of the fly in influencing the amount of virus recovered during the experiment. Statistical analysis showed that the amount of virus inoculated was less important than the duration of incubation of the insect in determining the titer. Preliminary results, using wild-caught culicoid flies, indicate that they are as susceptible to the intrathoracic inoculation of bluetongue virus as colony flies have been shown to be.

The Viremia of Sheep given a Previous Oral Enhancing Dosage of Bluetongue Virus. Improvements in bluetongue virus titrations made directly from sheep blood in embryonating chicken eggs made it possible to obtain immediate virus titers. The procedure utilized 7-day-old chicken embryos injected with the treated bluetongue virus blood in an equal volume of anticoagulant preservative solution. Treatment of the virus blood included the addition of 15 mg. lipase per 20 ml blood virus in anticoagulant preservative solution and sonification. Six eggs were inoculated, via the yolk sac, per dilution, held for 10 days, and the LD_{50} calculated by the standard Reed and Muench method.

Six sheep were infected with BT-262 virus during the first week of July, August, September, October, and November for a total of 30 principal sheep. Two additional sheep served as non-infected temperature control sheep. Three of the 6 principal sheep, for each month, were given a previous oral enhancing inoculum of 4 ml of blood virus in anticoagulent preservative solution daily for 10 days and then inoculated intradermally with the same blood virus 15 days after oral administration. Thus, all 6 principal sheep were inoculated intradermally on the same day.

The average peak bluetongue virus activity, measured by the total number of bluetongue virus chicken embryo mortalities, occurred on DAI-5 (days after inoculation). The bulk of the virus activity occurred on DAI-3 through DAI-10, with intermittent detectable virus present as early as DAI-1 and as late as DAI-21. The peak individual sheep virus titers, expressed as the $\log_{10}$ titer LD_{50} per 1 ml blood, ranged from 2.3 to 4.3. The blood virus collected in September gave the highest and most consistent virus titers. The highest virus titer was in an orally enhanced sheep, and a graphic correlation of the average peak bluetongue virus activity between the two principal groups demonstrated that the orally enhanced group had the higher virus activity. In addition, the orally enhanced group, in general, had the more severe bluetongue clinical responses. Three of the 30 principal sheep died and all three were orally enhanced sheep.

The sensitivity of bluetongue virus titrations was markedly increased by inoculating 9-day-old embryonating chicken eggs intravascularly. A comparison of the peak viremia for each principal sheep was compared by the two routes of assay inoculation. This comparison was made after 3 to 5 months storage at 4 C and gave an average intravenous 3 log increase in titer over the average yolk sac titer. There was an average of 1 log decrease in virus titer between the original versus the yolk sac titers after storage. The highest intravenous average virus titer was for the month of November with a 6.46 log value. The second highest was September with a 5.95 value.

An immediate assay comparison via the two routes of egg inoculation was conducted on bluetongue infected blood obtained from 3 sheep. The results demonstrated that the intravenous route of inoculation was consistently the more sensitive method for assaying virus. A 2 to 3 log increase of virus titer occurred during the height of the viremia period.
(Denver, Colorado) (ADP a3-5)

B. Vibriosis in Sheep

In work under a cooperative agreement with the Colorado Agricultural Experiment Station, Fort Collins, a study was made to determine the duration of immunity against ovine vibriosis. It began in November 1963, by vaccination of a group of yearling ewes prior to breeding. Vaccination was accomplished by giving a single 5 cc. subcutaneous injection of formalin-killed Vibrio fetus serotype I and serotype V organisms, mineral oil adjuvant, bivalent bacterin. Unvaccinated yearling ewes were maintained as controls.

This year, 14 of 22 nonvaccinated immunity challenge control ewes aborted, while no abortions occurred in 19 vaccinated ewes when the immunity of ewes of both lots was challenged with the combined V. fetus serotype I and serotype V culture challenge given during advanced gestation. From data obtained in this experiment, ewes vaccinated as yearlings demonstrated solid immunity against virulent V. fetus serotype I and serotype V organisms when their immunity was challenged at 3 years of age during their second gestation. (Fort Collins, Colorado)

In cooperation with the Montana Agricultural Experiment Station, Bozeman, work has continued on ovine vibriosis. The researchers at this station have expressed concern because many isolants of Vibrio fetus in their laboratory did not appear to fit into accepted serotypes for this organism. This year, with newly prepared serums and antigens against some of the troublesome isolants, 42 isolants were retyped. It now appears that the number of serotypes that will result from close application of their system will be greater than the number that was initially considered to have existed. Additional work by adsorption techniques and immunodiffusion studies is being conducted. (Bozeman, Montana)

In cooperation with the Utah Agricultural Experiment Station, Logan, the effect of vaccination of yearling replacement ewes to prevent vibriosis was studied for the fifth consecutive year in two herds with 2000 ewes each. In herd A no Vibrio fetus infection was detected but the agent of enzootic abortion (EAE) of ewes was isolated from 14 of the 33 abortions. Herd B was definitely exposed to V. fetus since 4 of the 33 abortions were positive for this infection; also fetuses from 12 abortions were infected with the EAE agent. Half of the abortions in this herd occurred among the few remaining, older, nonvaccinated ewes. Two ewes that were vaccinated the year previously had V. fetus infected lambs. The other V. fetus isolations were made from older ewes, one of which had normal lambs but a V. fetus infected placenta. Thus the vaccine was shown to provide effective protection against V. fetus infection. (Logan, Utah) (ADP a3-1)

C. Scrapie

Scrapie was first diagnosed in the United States several years ago. It is, however, not considered to be firmly established and efforts are continuing to eradicate it. Research has been conducted on this disease in Scotland and Great Britain for several years. The U. S. Department of Agriculture is supporting this research through PL 480 grants. In recent years, it has been determined that the disease is probably caused by a transmissible agent. The agent has, however, not been isolated nor characterized in detail. There is also increasing evidence that a certain genetic constitution is existent which determines susceptibility.

Additional information is required about the disease before eradication procedures may be improved. Significant progress has been made in that the disease has been transmitted to mice and in this species the incubation period is 4 months, contrasting to the incubation period in sheep of 4 to 36 months. The disease is being transmitted serially in mice and efforts are continuing to adapt the transmissible agent to other species of animals. Efforts are also being made to isolate the transmissible agent and adapt it to tissue cultures.

One of the things most needed and required before significant progress may be made on scrapie research is a rapid assay technique. Adapting the transmissible agent to tissue cultures appears to offer the most promise. A good biochemical approach is also being made to isolate the causative agent of scrapie from tissues from affected sheep, goats, and mice. In addition to the biochemical work under way, physicists are studying tissues from diseased animals using electronmicroscopy techniques in an effort to pinpoint the specific areas where the tissues are affected.
(PIADL) (ADP a3-3)

D. Paratuberculosis of Sheep

Johne's disease is an economic problem in some sheep flocks and an effective vaccine would greatly reduce losses from the disease. An immunization study covering a 6-year period was made with sheep to determine: 1) if sheep vaccinated as 2 to 4-week-old lambs were immune to Johne's disease, 2) if booster shots made the vaccine more effective, and 3) if the vaccine had any undesirable effects.

Used in the experiment were 54 nonvaccinated control lambs and 47 vaccinates. Thirteen of the controls developed the disease and only 1 of the vaccinates. It was found that the vaccine was safe to use and that booster shots were not required. (Ames, Iowa) (ADP a3-6)

E. Ulcerative Dermatosis in Sheep

In cooperation with the Colorado Agricultural Experiment Station, Fort Collins, the transmissibility of ovine ulcerative dermatosis was studied. The production of pustules in scarified skin of experimental sheep by inoculation with tissue from natural lesions was easily and repeatedly accomplished. Apparently the pathogenic agent contains several entities. Species of staphylococci in the pustular exudate are easily isolated and probably contribute to the cause of the pustule. Staphylococci, normally present on the skin of sheep, do not alone produce lesions as was indicated in the experiments by failure of the scarified but uninoculated skin to produce pustules. The precise role of staphylococci in lesion pathogenesis must be determined.

Experimentation of several years ago showed the presence of a virus in some lesions of ulcerative dermatosis. Under field conditions virus and staphylococci may interact to produce the lesion. The pathogenicity of the virus alone, the bacteria alone, and the virus and bacteria in combination, will be studied. (Fort Collins, Colorado) (ADP a3-4)

F. Toxicological Effects of Oxalate-Containing Plants

At the Division's Poisonous Plants Research Laboratory, Logan, Utah, in connection with this work, 16 lambs were divided into 8 pairs according to weight. One of each pair was fed a pellet containing 3.5% soluble oxalate while the other lamb received a similar pellet without oxalate. Water was given ad-lib, but the feed fed was regulated by the lamb of the pair eating the least.

The lambs were placed in metabolism cages for 84 days. Daily measurements were made of water and feed consumption and urine and fecal excretion. Blood samples were taken weekly for analysis.

Urine and fecal samples were collected daily for the first 5 days the lambs were on trial. Five-day total collections were made for the following 5 days at the half-way point and at the end. The urine was analyzed for

calcium, magnesium, phosphorus, sodium, potassium, chloride, and nitrogen. Urine pH and specific gravity was also determined. Fecal analysis will include nitrogen, calcium, magnesium, phosphorus, sodium, potassium, and chloride. The data collected remains to be critically analyzed.
(Logan, Utah) (ADP a3-7)

G. Identification of Teratogenic Agent in Veratrum californicum

In this work at the Division's Poisonous Plants Research Laboratory, Logan, Utah, 55 preparations from false hellebore roots (Veratrum californicum), a poisonous range plant, were prepared for biological assay in ewes to determine their ability to cause a congenital malformation in lambs.

It has been shown in previous studies that feeding the false hellebore plant to ewes on the 14th day after breeding caused their lambs' heads to be deformed. The 14th day was very specific for the teratogen as it would not affect the embryos when the ewes were fed on the 13th or 15th day of gestation. Through a process of elimination in feeding the various extracts to pregnant ewes on the 14th day of gestation, the causative agent, or agents, have been tentatively limited to four crystalline preparations. Two of the preparations were found to be glycosides and two parent alkamin steroidal alkaloids. (Logan, Utah) (ADP a3-8)

H. Chronic Toxicity of Herbicide Accumulation in Sheep Tissues.

At the Division's Poisonous Plants Research Laboratory, Logan, Utah, the toxicity and tissue residue of Atrazine and Monuron (soil sterilents) when ingested by sheep are being investigated.

Atrazine was lethal for all animals when fed at a rate of 30 mg/kg of body weight daily in 30 to 60 days. Fifteen mg/kg of body weight was toxic causing depressing and loss of body weight, but not lethal.

Monuron caused the death of two ewes and marked poisoning in three when fed at a rate of 75 mg/kg of body weight daily for 60 days. Thirty mg/kg of body weight has shown only slight toxic signs of loss of appetite and body weight. Studies to determine the tissue residue have not been completed.
(Logan, Utah) (ADP a3-9)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Vibriosis

Firehammer, B. D., and Hawkins, Jr., W. W. 1964. The Pathogenicity of Vibrio fetus Isolated from Ovine Bile. Cornell Vet., 54(2):308-314

Storz, J., Miner, M. L., and Marriott, M. E. 1964. Early Events in Vibrio fetus Infection of Ewes. Zentralblatt f. Veterinarmedizin II: 475-486

Paratuberculosis

Larsen, Aubrey B., Hawkins, Jr., William W., and Merkal, Richard S. 1965. Experimental Vaccination of Sheep against Johne's Disease. Amer. J. Vet. Res., 25:974-976.

Scrapie

Hunter, G. D., and Millson, G. C. 1964. Further experiments on the comparative potency of tissue extracts from mice infected with scrapie. Res. Vet. Sc., 5:2.

Hunter, G. D., and Millson, G. C. 1965. Studies on the heat stability and chromatographic behavior of the scrapie agent. J. Gen. Microbiol., 37:251-258.

Pattison, I. H. 1964. The spread of scrapie by contact between affected and healthy sheep, goats, or mice. Vet. Rec., 76:333.

Pattison, I. H., and Sansom, B. F. 1964. Dialysis of the scrapie agent. Res. Vet. Sci., 5:3.

Zlotnik, I., and Rennie, J. C. 1965. Experimental transmission of mouse passaged scrapie to goats, sheep, rats, and hamsters. J. Comp. Path., 75:147-157.

AREA NO. 4 - DISEASES AND PARASITES OF HORSES

Problem. Currently there are about 3,250,000 horses in the United States, valued at approximately $860 million. About one million of these are draft animals. Considerable numbers of horses and mules are still required for work on cattle ranches and as pack animals. The annual overall value of the horse industry has been estimated at about $1.5 billion. The horse may be an important link in epizootiology of animal diseases in general. Equine piroplasmosis is an acute, subacute, or chronic tick-borne disease of horses caused by protozoan parasites that was first recognized in this country in Florida in 1961. It is characterized by high fever, progressive anemia, jaundice, edema, extreme weakness and depression. Fatalities range from 5 to 50 percent of infected animals. This disease, now apparently well established in Florida, has extended into Georgia and poses a serious threat to the entire equine population in the southern United States. The disease is clinically indistinguishable from equine infectious anemia. Horses which have clinically recovered from piroplasmosis usually remain carriers of the disease and are a potential source of infection. African horse-sickness, a highly fatal disease of equines, that was confined to Africa has caused serious losses in the Middle East and parts of Asia in recent years.

USDA AND COOPERATIVE PROGRAM

The Department has a program involving biochemists, pathologists, protozoologists, and veterinarians working on equine piroplasmosis. In order to be prepared in the event of introduction of African horsesickness into the United States, the Plum Island Animal Disease Laboratory has obtained African horsesickness viruses and antiserums from South Africa. These materials are thus directly available for diagnostic and vaccine studies should the need arise.

The Federal scientific effort devoted to research in this area is 2.0 professional man-years. This effort is divided among sub-headings as follows:

Serological diagnosis, transmission, and control of equine piroplasmosis 2.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland (In cooperation with the Entomology Research Division), and under contracts with the University of Florida, Gainesville, and the University of Kentucky, Lexington.

PL 480 funds have been made available in Turkey for research on Gastrophilus pseudo-hemorrhoidalis (equine parasite) in Turkey, and for the study of the horsesickness virus.

PROGRAM OF STATE EXPERIMENT STATIONS

Workers in many states are continuing to develop new useful knowledge concerning the treatment and control of equine disease conditions.

Efforts are being made to study the anatomy and mechanics of bones, joints, ligaments and tendons in relation to the occurrence of lameness and certain skeletal anomalies. Some of these basic studies complement and extend knowledge concerning athletic injuries in man and skeletal problems in all animals.

Vaccines for the prevention of viral rhinopneumonitis, equine viral arteritis and influenza are being evaluated under laboratory and field conditions. Studies are continuing on the development of procedures for the diagnosis and prevention of respiratory diseases of horses.

Much effort is being made to develop and evaluate more effective drugs and procedures for the prevention and control of equine parasites. Parasite-free foals are being employed in gaining new knowledge concerning the disease-producing mechanism produced by certain internal parasites.

In addition, attention is being given to other conditions such as piroplasmosis, colitis, infertility, and diseases of the newborn.

The total State scientific effort devoted to equine disease research is 14.5 professional man-years annually.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Equine Piroplasmosis

At the Beltsville Parasitological Laboratory, investigations on equine piroplasmosis have been continued in the areas of research on characterization and transmission of the protozoa Babesia caballi that causes the disease, and the development of a standard antigen for use in serological diagnostic tests. The existence in the United States of Babesia equi, another etiologic protozoan agent for equine piroplasmosis, was positively confirmed. (BPL)

Research studies are being conducted at the Agricultural Experiment Station, University of Florida at Gainesville, under a contract with the USDA on investigations designed to evaluate chemotherapeutic methods of prevention, treatment and eradication of piroplasmosis in horses. To date a total of 132 cases have been treated, 41 of these being field cases. Nine drugs have been used and three, Berenil, Diampron, and Phenamidine, were useful in elimination of the "carrier" state. (Florida)

Studies are under way at the Agricultural Experiment Station, University of Kentucky at Lexington, under a contract with the USDA, on investigations designed to develop antigenic material for use in diagnostic tests for equine piroplasmosis. (Kentucky) (ADP b6-13C)

B. Gastrophilus pseudohemorrhoidalis (equine parasite) in Turkey, etc.

Under a PL 480 Grant to the Veterinary Faculty, Ankara University, Ankara, Turkey, research investigations are in progress on the distribution, life cycle, treatment and control of the equine parasite Gastrophilus hemorrhoidalis. A total of 905 equines were examined for the parasite larvae and of these 675 horses and 225 donkeys were found to harbor the larvae of different Gastrophilus species. Eighty-two third instar larvae were obtained which had the characteristic body structure of the G. hemorrhoidalis. Eleven of them hatched into flies but only two reached maturity.

No destructive effect on the incubating eggs of the Gastrophilus species was observed following the application of a 1% solution of Isotox, a 0.01% solution of Asuntol, or a 10% solution of Neguvon to the skin of horses. The oral administration of Neguvon, 35 mg./kg. body weight, was found to be effective for control of second and third instar larvae of the Gastrophilus species that were not attached to the wall of the rectum. (A22-ADP-4)

C. Horse Sickness

Under a PL 480 Grant to the Veterinary Faculty, University of Ankara, Ankara, Turkey, studies of the horse sickness virus (HSV) in tissue culture, its serological and immunological characteristics, and vectors, have continued. The report shows that tissue cultures were prepared from sheep, calf, dog, donkey and goat kidney cells, and monkey cells. The horse sickness virus propagated in dog, donkey and monkey kidney cells, but not in sheep, calf and goat kidney cells. Seven virus strains were passaged 10 times and did not show any loss of capability to infect cells. Tenth passage horse sickness virus in tissue culture fluids were tested for hemagglutination with erythrocytes of guinea pig, rabbit, goat, sheep, horse, donkey, and calf. The virus showed low hemagglutination only with erythrocytes of rabbit and horse. (A22-ADP-7)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

None

AREA NO. 5 - INFECTIOUS AND NON-INFECTIOUS DISEASES OF POULTRY

Problem. Annual losses from infectious and non-infectious diseases of poultry, exclusive of parasitisms, are estimated to be at least $200 million. Continued and expanded basic and applied research are essential to aid in reducing these losses, which inevitably affect cost to the consumer. Added to the initial losses from mortality, reduced weight gains, poor feed utilization, decreased egg production, and lowered quality, are the final losses occasioned by condemnations at dressing plants. United States turkey growers in particular, are faced with a new problem in that a newly discovered infection with a different strain of Mycoplasma is widespread in flocks throughout the country. Resulting condemnation losses at slaughter are often great. The problem is to keep abreast of changing conditions in the field, which present increasingly complex problems requiring basic information.

USDA AND COOPERATIVE PROGRAM

The Department has a long-term program involving biochemists, microbiologists, pathologists, and veterinarians engaged in both basic studies and the application of known principles to the solution of infectious and non-infectious diseases of poultry. Research is being conducted on the diseases at the following locations.

The Federal scientific effort devoted to research in this area totals 17.5 professional man-years. This effort is applied as follows:

Ornithosis 2.7 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the Universities of California and Minnesota, and the Agricultural Experiment Stations of Oregon and Texas.

Salmonellosis 1.0 at the National Animal Disease Laboratory, Ames, Iowa, and the Southeast Poultry Research Laboratory, Athens, Georgia.

Pasteurellosis 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

Chronic Respiratory Disease Complex 6.3 at the National Animal Disease Laboratory, Ames, Iowa, the Southeast Poultry Research Laboratory, Athens, Georgia, and under cooperative agreements with the Agricultural Experiment Stations of Connecticut, Delaware, Georgia, Massachusetts, New York, North Carolina, Texas, Virginia, and Wisconsin, and with the University of Minnesota.

Newcastle Disease 3.2 at the National Animal Disease Laboratory, Ames, Iowa, the Southeast Poultry Research Laboratory, Athens, Georgia, and under cooperative agreements with the University of Maine and the Wisconsin Agricultural Experiment Station, and under a PL 480 Grant to the Institute for Veterinary Research, Pulawy, Poland.

Leukosis 0.3 under cooperative agreement with the Regional Poultry Research Laboratory, USDA, East Lansing, Michigan.

Infectious Bronchitis 2.0 at the National Animal Disease Laboratory, Ames, Iowa, and the Southeast Poultry Research Laboratory, Athens, Georgia.

PROGRAM OF STATE EXPERIMENT STATIONS

Major emphasis is being placed by the State Stations on the respiratory disease complex of poultry (airsacculitis) with particular attention being given to chronic respiratory disease (CRD). Two regional research projects (NE-5 and NC-65) coordinate the work of 20 States and the Department on the respiratory disease complex with the principal effort centered on means for control or eradication of CRD. The role of environment as it affects outbreaks of the disease is being studied and efforts are being made to improve diagnostic materials to locate CRD carriers. Control by means of antibiotic therapy is under investigation and the effectiveness and limitations in using virulent vaccines are being determined by a number of States. Basic investigations are under way to characterize the physical and biological properties of Newcastle Disease, infectious bronchitis, laryngotracheitis and fowl pox viruses.

Other research is concentrated on the important problem of leukosis to determine means of transmission, practical methods for laboratory diagnosis of virus carriers, and effective methods for prevention. Increasing emphasis is being placed on salmonellosis to determine the sources of infection, means of transmission, and practical methods for elimination from poultry products. Basic studies are in progress on the factors affecting resistance of poultry to this disease.

Research also is in progress to provide improved control methods for conditions such as coccidiosis, ascariasis, ornithosis, dissecting aneurysm, Gumboro Disease, mycotic infection, and other important poultry problems.

The States are allotting 93.2 professional man-years to poultry disease research.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Ornithosis

At the National Animal Disease Laboratory, Ames, Iowa, basic research is in progress on this problem. It is directed toward relationships between psittacosis-group agents found in wild birds and those found in domestic birds and mammals. In order to understand the relationships between various psittacosis-group agents that affect domestic animals, four strains of these agents were studied to see how they affected laboratory animals, wild and domestic birds, and a domestic mammal. The strains studied were those causing turkey ornithosis, pigeon ornithosis, lamb abortion and lamb polyarthritis. These strains were inoculated into mice, guinea pigs, chicken

and turkey embryos, pigeons, parakeets, sparrows, turkeys, and sheep. Lethal and infectious endpoints were determined for each strain in each of the species. These determinations revealed specific pathogenicity differences between the strains sufficient to be readily identified in the laboratory on the basis of the effects they cause in just three of the species - mice, guinea pigs, and pigeons.

Of epizootiologic interest were the observations that the virulent turkey strain caused pneumonia in lambs, the pigeon strain produced severe air-sacculitis in turkeys and both sheep strains caused disease in turkeys. The significance of these results is that it may be possible for these agents to transfer between hosts, thus possibly to establish intermediate carrier hosts which in turn would perpetuate the disease agents.

(NADL) (ADP a5-20)

At the University of California, Davis, cooperative work continued. Over 212 chickens of various ages were inoculated by various routes with the C-1 strain of ornithosis agent. No signs were observed in any of the chickens and only two birds presented lesions of pericarditis and plastic exudates over the livers. The virus could be isolated from the blood, saliva, cloaca, liver, and lung for various periods of time beginning 72 hours after exposure. The virus was isolated from the lung and saliva of asymptomatic chickens after a period of 7 weeks, the longest period tested. Complement-fixing antibodies were dependent upon the amount of virus in the inoculum. It was concluded that chickens are resistant hosts but potentially can be carriers of the infection. Stress, such as exposure to CO_2 atmospheres following inoculation, had some influence in the course of infection.

Biochemical analysis of the CF fraction of the antigen prepared from the Herellea-like bacterium showed it to be extremely stable to temperature, pH, and could be salted out with ammonium sulphate. The fraction was demonstrated by chromatography to have a peak at 260 mu.

(California) (ADP a5-20)

In cooperative studies at the University of Minnesota, St. Paul, routine serologic surveillance, involving some 4,291 samples from 119 turkey flocks in Minnesota and Wisconsin, indicates that about 20% of the flocks tested for ornithosis antibodies gave significant reactions. Individual turkey samples submitted from Iowa, not considered flocks, yielded reactions regarded as suspicious.

A small number of samples obtained from pigeons, chickens, and geese, were considered negative. Sera obtained from pheasants (195 samples) revealed some reactions in the suspect class. Virus isolation attempts from natural cases have been unsuccessful during the year.

A pilot study utilizing bovine sera in the complement fixation test with ornithosis virus antigens indicates a high percentage of reactions at low serum dilutions. The significance of these reactions is unknown.

A synthetic medium has been developed for propagating the Herellea-like bacterium, components of which cross-react with the ornithosis virus. (Minnesota) (ADP a5-20)

At the Texas Agricultural Experiment Station, College Station, histopathologic lesions of meningitis or meningoencephalitis, occurred in 5 of 20 turkeys infected by exposure to sheep experimentally infected with virulent ornithosis virus. This is the first report of central nervous system lesions from this disease in turkeys.

Antigen production was shifted to cell culture methods to give a more specific antigen than the older yolk sac method.

A new highly specific agglutination procedure was developed for use with turkey serums. This procedure is much more rapidly run without elaborate laboratory equipment.

The Jo strain of ornithosis virus was used to experimentally infect chickens. The chickens demonstrated clinical signs up to 3 weeks, but not after. A Psittacosis - LGV group virus was isolated from, and studied in, lambs. Serological evidences indicate this agent to be widespread in lambs in Texas. (Texas) (ADP a5-20)

B. Chronic Respiratory Disease Complex

At the National Animal Disease Laboratory, Ames, Iowa, the following work was accomplished:

In outbreaks of Mycoplasma gallisepticum infection in poultry, variations in severity have been observed, as have variations in the incidence of the forms, such as sinusitis or airsacculitis, which occur. These variations may be the result of within-species variation of the causative organism. The following study describes the effects of a particular isolate of M. gallisepticum (strain 1010) on turkeys and compares the effects of intrasinus exposure of this isolate with another.

The serologic, pathologic, and symptomatic responses of adult male Beltsville White turkeys exposed by intratracheal inoculation, intrasinus inoculation, intranasal inoculation, and by contact with inoculated birds to M. gallisepticum isolate 1010 were compared. Signs of infection varied among the groups with tracheitis occurring only in those exposed intratracheally or by contact, and sinusitis occurring only in those exposed via the sinuses. Airsac lesions did not differ appreciably among the groups in severity or incidence.

Serologic response was initially higher in turkeys exposed by intratracheal or intrasinus inoculation. The hemagglutination-inhibition test was found to be more sensitive than the tube agglutination test for detecting antibodies against M. gallisepticum.

Comparison of the effects of intrasinus exposure of turkeys with M. gallisepticum isolate 1010 and M. gallisepticum isolate 1150, revealed isolate differences. Isolate 1010 caused a slightly higher incidence of sinusitis, but a much lower incidence of tracheitis than isolate 1150. Air-sac lesions did not differ in incidence or severity. The differences observed indicate differences in tissue predilection of the isolates.
(NADL) (ADP a5-21)

At the Southeast Poultry Research Laboratory, Athens, Georgia, a detailed study was conducted on birds exposed intranasally to Mycoplasma gallisepticum (R strain) under field conditions, at 38 days of age. Groups of exposed birds were transferred from the field at 23 weeks of age and placed in separate laboratory isolations. Subsequent isolation, contact exposure, challenge and serological tests demonstrated that 1) vaccinated birds were shedding Mycoplasma from 29-43 weeks of age, as evidenced by tracheal isolations and spread to susceptible contact birds; 2) vaccinated birds were susceptible to homologous and heterologous challenges at 28 weeks of age, and 3) progeny of exposed birds were not free of Mycoplasma, since isolations were made from day-old chicks and 18-day embryonated eggs.
(SEPRL) (ADP a5-17)

A method for culturing embryonic chick lung cells was developed and infectious bronchitis virus strain 42 has been adapted to these cells. This permits the study in vitro of infectious bronchitis virus (IBV) in epithelial cells of respiratory tract origin. This virus propagated in these cells is of high titer, is cytopathogenic, and will produce plaques. Another strain of IBV(317), has been carried through 10 serial passages in these cells. This represents the highest passage level in tissue culture of a field strain of IBV from which virus has been recovered. The results support the belief that field strains can be adapted to tissue culture system, and that this system can be used as a tool to investigate the virus.

Growth curves are being constructed in order to determine if there are any differences in the growth characteristics of the various IBV strains. The results of 4 such curves show that the egg adapted strain reaches its peak titer about 5 hours prior to the field strains. The curves produced by 3 field strains are identical.

A procedure for utilizing a serum neutralization test was developed. This procedure, in which a constant virus dose is run against varying dilutions of serum, is expected to be more sensitive in showing antigenic differences in the virus. During the course of these investigations, the virus was found to be unstable at room temperatures. A dilution medium was developed to overcome this difficulty since it is imperative that virus stability be maintained. (SEPRL) (ADP a5-23)

In cooperation with the Animal Husbandry Research Division, work was initiated to establish flocks resistant and susceptible to Newcastle disease virus (NDV). Athens Randombred stock has been used in this work. A mean

flock titer for NDV was first determined. Titers were then determined in eggs and chicks from individual hens. Correlations run on data from these trials were not high (r.2). However, a beginning selection has been made, based on families which were most consistently represented in either the high group or the low group. (SEPRL) (ADP a5-18)

At the Connecticut Agricultural Experiment Station, Storrs, under a cooperative agreement with the USDA, researchers ran experiments to compare the performance of birds vaccinated at 8-12 weeks of age with live pathogenic Mycoplasma gallisepticum (MG) with nonvaccinated birds which acquired Chronic Respiratory Disease (CRD) naturally. Results showed that vaccinated birds performed much better in regard to egg production and mortality.

In areas where CRD is endemic, vaccination of flocks, particularly those used as a source of market eggs, appears to be highly desirable until such time that an eradication program can be implemented.

Birds vaccinated at 8-10 weeks of age and examined 18 months later showed high titers to MG. They were also resistant to challenge.

Progeny from vaccinated dams were serologically tested for MG at 8 weeks of age. Of 229 groups of birds (30 birds per group), four groups were positive and 225 were negative. The low rate of transmission suggests that vaccination and subsequent periodic testing of progeny flocks to weed out the positives can be an effective means to eventual eradication of MG in poultry.

A completely synthetic medium was developed for Mycoplasma laidlawii B. This is the first successful attempt to grow any Mycoplasma in a chemically synthetic medium. (Connecticut) (ADP a5-17)

At the Delaware Agricultural Experiment Station, Newark, cooperative research has been directed toward the formulation and operation of an area plan for the control of Chronic Respiratory Disease (CRD). The two States of Delaware and Maryland, as well as the State of Virginia to a lesser extent, are involved. The State Boards of Agriculture are cooperating and the poultry industry association (Delmarva Poultry Industry, Inc.), is giving enthusiastic support. The program is one of controlled exposure using an attenuated broth culture as the vaccine at 8-10 weeks of age on infected replacement flocks. Prevaccination blood titers and a series of postvaccination titers are used as a partial criteria of the program's success. In addition the health of the broiler chicks produced, their weight, feed conversion, condemnation rate, cost of producing, and other factors are being included in the evaluation. Also, the disease history of the vaccinated birds and the hatchability of their eggs are other items included in the test. By the summer of 1966, the results of the tests should indicate its feasibility. (Delaware) (ADP a5-17)

In cooperative work at the Georgia Agricultural Experiment Station, Athens, preliminary studies show that it may be possible to increase the resistance of young chickens to Mycoplasma gallisepticum infection by intramuscular injection of living organisms. There are, however, some factors which need to be affirmed before such a procedure could be considered practical. Furthermore, some serological test more sensitive than the agglutination test needs to be developed to determine the immune response in chickens.

(Georgia) (ADP a5-17)

At the Massachusetts Agricultural Experiment Station, Amherst, cooperative studies gave the following results:

Transmission of Chronic Respiratory Disease (CRD) through cohabitation of CRD serologically positive birds and susceptible birds may occur under certain conditions after a prolonged exposure period. CRD serologically positive birds may be true carriers and may remain carriers for an extended period after the initial disease outbreak. Under certain conditions, shedding may occur which may result in transmission to susceptible contacts. However, in some instances no transmission is apparent, even after prolonged exposure periods. These naturally infected serologically positive birds may yield M. gallisepticum that is capable of producing the disease in artificially inoculated birds and embryos up to 24 months after the disease outbreak.

Birds were experimentally injected with M. gallisepticum. Antibody titers persisted in all but one of the birds after inoculation. The rapid-serum-plate, tube agglutination, and hemagglutination-inhibition tests were in general agreement. Doubtful, unexplained tube agglutination and rapid-serum-plate reactions occasionally occurred in the control groups. The nonspecific hemagglutination-inhibition titers obtained in the control group when the antigen prepared by USDA procedures was titrated against untreated serum samples, could be removed completely when the serum samples were treated with sodium meta-periodate and receptor-destroying enzyme of Vibrio cholerae. This nonspecific reaction did not occur with live broth antigens. M. gallisepticum was reisolated from the respiratory tract up to 5 weeks post-inoculation. After this period, isolation was sporadic with recovery of the organism on the 10th, 11th, 14th, and 19th weeks. This work is still in progress.

Varying degrees of agglutination of M. gallisepticum S6 antigen may be detected in sera from day-old chicks produced by positive dams. Parental agglutinins may be detected in progeny of exposed dams within a short time after the development of CRD agglutinins in the adult birds.

Birds that have undergone a natural outbreak of CRD may exhibit a wide variation in serologic titers to the tube agglutination and hemagglutination-inhibition test procedures. The rapid-serum-plate test gave the most consistent positive results. Samples collected from known negative birds show close agreement by the rapid-serum-plate, tube agglutination, and hemagglutination-inhibition tests.

Attempts to eradicate CRD from two breeding flocks through management and tylosin medication of the dams and progeny have yielded encouraging and promising results.

CRD-free stock can be produced, maintained, and reproduced if adequate sanitation and preventive practices and reliable testing methods are employed. The majority of negative premises continue to remain negative on successive years. The above statements are based on 9 years of observations.
(Massachusetts) (ADP a5-17)

In cooperative research work at the University of Minnesota, St. Paul, egg dipping and water medication with tylan were used to produce a Mycoplasma gallisepticum-free flock of chickens. This flock was blood tested at 5, 10, 22, 33, and 48 weeks and remained serologically negative to the serum plate and hemagglutination-inhibition (HI) tests for M. gallisepticum.

Two small flocks of "Mycoplasma Free" turkeys are being maintained at the Rosemount Experiment Station. Progeny of one of these flocks is in its third season and the other in its second. Poults from these flocks have remained free of any cultivatable Mycoplasma and are free of the day-old airsacculitis associated with these organisms.

The factors affecting the efficiency of tylan absorption into turkey eggs were studied. Time, temperature, concentration of tylan and the effect of surface reducing agents were studied. A time of 15 minutes with a 35°F temperature differential between the dip solution and the eggs, and 3,000 ppm have been selected as the most practical for field use. Surface reducing agents have little effect at this concentration.

A salvage program for two valuable turkey breeding flocks infected with Mycoplasma gallisepticum is being tried. This program consists of treating the infected flock (both hens and toms). The eggs from these flocks are dipped in tylan. The poults are being water medicated for 5 days at one day of age. The poults from these flocks are inspected periodically for signs of respiratory disease and tests are conducted for M. gallisepticum antibodies. The egg transmission of the "N" strain of avian Mycoplasma was studied. Two experiments were conducted in an effort to determine the nature of the egg transmission of this strain. The rate of transmission appears to increase as the production period increases. The adult hen is able to localize the infection in the upper respiratory tract. Consequently no Mycoplasma could be isolated from the air sacs of artificially infected hens by the aerosol inhalation method. Further studies on the association of the length of time in production with the transmission rate are being conducted.
(Minnesota) (ADP a5-17)

Field investigations were conducted on thirty-one clinical outbreaks of infectious sinusitis in turkeys during the past year. The major reason for the outbreaks was egg transmission from an infected breeder flock (59%). The next important source was exposure from infected chickens (15%). Imported hatching eggs and lateral transmission also were important causes of outbreaks.

Studies were continued on the effect of environmental conditions on the airsacculitis syndrome in turkeys. Three experiments were conducted in an effort to raise "Mycoplasma Free" fryer-roaster turkey flocks. The eggs were dipped in varying concentrations of tylan and in one experiment the day-old poults were water medicated. The airsacculitis observed at the processing plants was very minimal in all three flocks. However, a Mycoplasma free status was not attained.

Air samples were taken in the environmental turkey buildings throughout the last two experiments. The counts of bacteria per cubic foot of air increase very sharply the first four weeks. They were found to be somewhat higher during the winter months. This may result from a reduced air flow as a result of the low ambient temperatures.

The voluntary control program for Mycoplasma gallisepticum which consists of a 100% testing by all turkey breeders continues to be effective in minimizing this infection in Minnesota turkeys. Only 105 birds of 659,928 tested were submitted to the University for further laboratory analysis.

A survey of the incidence of the "H" serotype of Mycoplasma in Minnesota turkeys was conducted. Sixty-four percent of all samples tested reacted to the serum plate test antigen developed at the University of Minnesota. (Minnesota) (ADP a5-21)

In cooperative studies at the New York Agricultural Experiment Station, Ithaca, researchers found that the failure of a live pathogenic culture of Mycoplasma gallisepticum to induce 100% protection against egg transmission indicated that this method of immunization into the air sac by itself cannot be used to initiate production of disease-free progeny. Vaccination by intranasal instillation with live cultures likewise did not afford complete protection after challenge. The vaccination itself induced egg transmission in some instances.

An attempt to produce clean progeny from infected breeding stock by dipping the eggs in Tylosin solution has given encouraging results thus far. Special care must be taken to avoid adventitious infection during the growing and laying period on the farm. No evidence of infection has been found in 9 dipped hatches. The undipped eggs from 3 of these hatches yield infection.

Under experimental conditions, brief (4 days) contact exposure to infected birds or exposure to contaminated premises for 15 weeks failed to induce M. gallisepticum infection in clean chickens.

A simple medium incorporating coagulated egg yolk has been found highly effective for growing most avian mycoplasma. (New York) (ADP a5-17)

In cooperative research at the North Carolina Agricultural Experiment Station, Raleigh, extensive field experimentation has been undertaken in an evaluation of experimental planned infection (EPI) in the immunization of chickens against Mycoplasma gallisepticum, employing live culture inocula. To date individual inoculations have been made on slightly more than 900,000 broiler-type replacement pullets between 8 and 18 weeks of age via the intranasal or posterior thoracic air sac routes. One-tenth ml. of broth culture of the California Chick-F isolate has been used on all flocks. Approximately 1% of the birds in candidate flocks are tested (serum-plate) as a means of serologically classifying flocks prior to inoculation.

Clinical symptoms following EPI inoculations have been relatively insignificant. However, some flocks have required medication. Several flocks are now under investigation to determine the validity of suspected concurrent virus respiratory infections. Two flocks subjected to laryngotracheitis vaccination approximately 2 weeks post EPI, developed typical air-sac disease symptoms and lesions and required medication. Progeny data from EPI flocks is not sufficient at this stage of the experimentation to justify application.

Two hundred and fifty one flocks have been serologically tested at least once for M. gallisepticum antibodies, of which 82% were positive as a result of natural exposure. Many serologically positive flocks revealed that the rate of advancement to a 100% positive status was slow and the degree of agglutination reaction was only moderate. Twenty M. gallisepticum-clean parent and grandparent flocks have been negative on all tests to date. However, some of these flocks are not yet sexually mature.

Cross agglutination between M. gallisepticum antigen and M. synoviae antibodies is an important consideration in flock classification. It is essential that M. gallisepticum-clean flocks yielding weak positive reactors be tested for M. synoviae before the clean status is revoked. The need for a suitable M. synoviae antigen becomes obvious.

(North Carolina) (ADP a5-17)

At the Texas Agricultural Experiment Station, College Station, in cooperative studies on infectious sinusitis eradication, 227 breeder flocks, representing 218,949 turkeys, participated in the 1964 M. gallisepticum eradication program. Five M. gallisepticum-infected flocks were identified and marketed.

In work with M. gallisepticum antigens, cooperative studies with the Animal Disease Eradication Division of the USDA were continued and standard production and testing protocols for M. gallisepticum plate, tube and hemagglutination-inhibition (HI) antigens were developed. These findings were made available to industry and at least one commercial firm is now producing antigen.

Condemnation studies revealed that 55.5 percent of turkey condemnation in Texas are due to airsacculitis. However, M. gallisepticum infection was responsible for only 83 of 633 birds condemned in this category. This

confirms the value of the infectious sinusitis eradication program. However, it does point out the need for additional research on causes of airsacculitis, particularly fowl cholera, aspergillosis, and other undetermined causes.

Quail bronchitis virus was shown to have little potential as a "triggering mechanism" for air sac syndrome. However, evidence was obtained that some strains might be more pathogenic to broilers than heretofore supposed. (Texas) (ADP a5-17)

Cooperative work at the Virginia Agricultural Experiment Station, Blacksburg, has shown that a disease of chickens characterized by reduced weight gain and retained caseous yolk sacs was reproduced by dipping incubating eggs in a suspension of 0103 serotype Escherichia coli obtained from a field flock affected with a similar condition.

Social stress was produced in chickens between 56 and 70 days of age by moving male White Leghorn chickens into cages with other birds according to a schedule which kept contact with previously encountered birds to a minimum. At the end of the 2-week social stress period, the stressed birds were more resistant to pathogenic strains of Escherichia coli inoculated via the air sac than the unstressed controls. Social stress did not increase resistance to birds similarly inoculated with Mycoplasma gallisepticum. (Virginia) (ADP a5-17)

In cooperative research studies at the Wisconsin Agricultural Experiment Station, Madison, chickens exposed artificially to poultry house dust in an environmental chamber showed no gross or microscopic signs of damage to the respiratory tract after 6 hours of continuous exposure, or 2-hour exposures for 8 consecutive days. Chickens exposed "naturally" to ammonia carbon dioxide, and dust in the environment of a poultry house for 6 days, showed some loss of cilia from the epithelium of the upper portion of the trachea and the turbinates and an increase in mucus secreting goblet cells. Dust particles were present in the macrophages of the lungs. When these chickens were exposed to a secondary stress in the form of a respiratory infection initiated by an aerosol of Newcastle Disease virus, there was, as compared to control birds receiving only the NDV aerosol, a possible shortening of the mean death time and possible increased percent mortality.

Attempts have been made to develop a serological test which will overcome some of the disadvantages of the conventional colony inhibition test used for serological classification of the mycoplasma. A test in which porous paper strips are used as a vehicle for interaction between the unknown strains and known antisera has shown some advantages and some disadvantages. Some strains of the organism do not give good reactions in early culture passages. (Wisconsin) (ADP a5-21)

C. Salmonellosis

At the Southeast Poultry Research Laboratory, Athens, Georgia, studies have been completed on the flagellar antigenic balance and hemagglutination properties of 565 cultures of Salmonella typhimurium isolated from avian sources in the United States. Approximately 95% of these cultures have been demonstrated to produce hemagglutination of avian red blood cells. Based on naturally occurring high levels of phase 1 and phase 2 flagellar antigens, 33 cultures of S. typhimurium have been selected for further antigen preparation and growth studies. These cultures are being subjected to detailed single-factor somatic antigenic analysis in further characterizing their sensitivity and antigenic balance for use in detecting serological reactors to S. typhimurium agglutination tests.

Techniques have been developed and are presently under further detailed study for sampling microbial penetration through the shell and shell membranes of chicken eggs under varying conditions of temperature and humidity. Salmonella typhimurium is being used as the test organism. These methods involve the mechanical separation of the eggshell and its membranes prior to exposing selected areas of the shell surface to known numbers of bacterial cells. These studies are being extended to evaluate the effects of egg treatments such as formaldehyde fumigation and egg washing on the penetration patterns of Salmonella organisms. Preliminary studies have indicated that the penetration rate of these bacteria through the shell surface is much more rapid than previously accepted and described using less refined methods of sampling. (SEPRL) (ADP a5-2(Rev.)

D. Pasteurellosis

At the National Animal Disease Laboratory, Ames, Iowa, in studying the host-parasite relation of fowl cholera, experiments were designed to study the action of the organism and the reaction of the host. The most pronounced and significant lesion of acute fowl cholera has been found to be generalized passive hyperemia which resulted from cardiac insufficiency, atony of veins and capillaries. These lesions were indicative of the syndrome of shock which is often attributed to the action of endotoxins.

During the past year studies were made of the structure and characteristics of P. multocida as related to its pathogenesis. Particulate antigens were isolated from noncapsulate avirulent cells of two immunogenically distinct strains (X-73 and P-1059) of P. multocida. They possessed many of the properties of endotoxins. Almost 100% of chickens given sublethal injections of these antigens were protected when challenged with live organisms which killed 100% of the controls. The same degree of protection was obtained in mice. Injection of various amounts of the antigens into mice, rabbits, or chickens produced moderate to severe toxic effects such as depression and diarrhea. Death frequently followed. Chickens had the same signs after injection as has been observed with cases of acute fowl cholera.

It is concluded that in the pathogenesis of fowl cholera, the ability of P. multocida to form a capsule is significant. The capsule allows the organism to grow and produce in the host an endotoxin which is responsible for the signs observed in acute fowl cholera. A vaccine prepared with the endotoxin will stimulate immunity against the natural disease.

The immune response stimulated by each strain of P. multocida used in fowl cholera vaccines can be determined by challenging groups of vaccinated birds. It would be advantageous to have a serologic test that would indicate the immune response in birds stimulated by these organisms and differentiate immunogenic strains. Experiments were designed to compare the immune response of chickens and turkeys to 2 known and 2 unidentified immunogenic strains, and to compare the passive-immunity test, agar double-diffusion test and serum-plate agglutination test to the immune responses.

The immune status of chickens and turkeys to P. multocida could not be determined by these 3 tests. Immune and hyperimmune chicken serum did not induce passive immunity in mice. Strains of P. multocida that differed in their fermentation, agglutination, immunogenic, and pathogenic characteristics could not be differentiated on the basis of the agar double-diffusion test. The agglutination test did not clearly indicate the immune status of chickens and turkeys that were vaccinated, tested serologically and exposed with homologous cultures. There was very little cross-agglutination with some cultures, but there was cross-immunity. Vaccines containing only one strain of P. multocida gave better immunity in turkeys to homologous challenge than bivalent or trivalent vaccines. However, all vaccines gave good protection in chickens, with the exception of those challenged intravenously. Turkeys were more susceptible to P. multocida than chickens, and mature chickens were more susceptible than young chickens.

It is apparent as a result of these studies that there is no complete correlation of the serologic, biochemic, immunogenic, or pathogenic methods of typing P. multocida and that these serologic tests cannot determine the immune status of chickens and turkeys.

Two strains of Pasteurella multocida (X-73 and P-1059) of avian origin are commonly used in fowl cholera vaccines in the United States. Heddleston reported that these strains differed in their immunogenic, serologic, pathogenic and biochemical properties. Namioka and Musata typed these two strains on the basis of capsular and somatic antigens and placed X-73 in serotype 5:A and P-1059 in serotype 8a:A. They also described a serotype 9:A which was isolated from a turkey in the United States, and a serotype 8:A which was very similar to 8a:A. They reported that serotypes 5:A, 8:A and 9:A did not cross-immunize. Since only 2 immunogenic types are included in fowl cholera vaccines in the United States, a comparative study of serotypes 9:A, 8a:A and 8:A was undertaken.

The serotypes could not be differentiated on the basis of immune response. Each isolate gave a high degree of immunity against homologous and heterologous challenge. On the basis of the tube-agglutination test and cross-absorption of immune chicken serums, serotypes 8a:A and 9:A were similar. Serotype 8:A contained an additional antigen (positive at 1:20) which was not detected with the serum plate test. Biochemical fermentations were similar with the exception that one strain did not ferment xylose. For the present, at least, only two serotypes of Namioka and Murata, 5:A (X-73) and 9:A (P-1059) are necessary in the production of fowl cholera stock vaccines in the United States. (NADL) (ADP a7-25)

E. Newcastle Disease

At the National Animal Disease Laboratory, Ames, Iowa, basic research on the Newcastle Disease problem is directed toward improving inactivated vaccine for susceptible chickens at various ages. (NADL) (ADP a5-18)

Cooperative research at the University of Maine, Orono, has been directed toward the prevention of Newcastle disease by the use of killed vaccine. The vaccine has proved very effective for control of the disease.

A Specific Pathogen Free (SPF) program has been conducted on broiler and breeder flocks during the past year. A rigid set of standards for isolation and husbandry are required to conform to the program. For the entire period of study at least 700,000 tests have been made on breeding hen flocks in an effort to eradicate S_6 PPLO. (Maine) (ADP a5-18)

Cooperative research at the Wisconsin Agricultural Experiment Station, Madison, was in four areas - 1) accession and identification of new isolates; 2) study of virus genetics; 3) study of pathogenesis, and 4) study of epizootiology.

In the first area, 14 cultures of Newcastle Disease virus (NDV) were received in the past year. Most of them brought important questions. The virulent culture recovered from a chicken in Maine resembled the virus used in producing the killed vaccine which the flock had received. The question was, were the two cultures the same? While identity can never be conclusively established, the plaque morphology, physical stability, biological properties in embryos and chickens were such that the culture could not be distinguished from the vaccine stock. The supplier suggested that the vaccinated flock was probably contaminated by an unknown contact with the nearby farm where the vaccine potency tests were conducted rather than by receiving un-inactivated virus in the vaccine.

Nine isolates that had been recovered from vaccinated chickens were supplied from Quebec. All these cultures contained Newcastle disease virus. The cultures differed in plaque morphology, embryo and chicken pathogenicity, and probably represent at least 3 strains. None of the cultures can be classed as exotic as enteric lesions are not produced in chickens.

A culture of Newcastle disease virus with very low pathogenicity for chicken embryos was recovered from a laryngotracheitis virus isolate obtained near Bakersfield, California. It was first believed to be Yucaipa virus. Two cultures have not yet been examined. One is an exotic strain isolated from Indonesian Cockatoos. The other is a strain of Newcastle disease virus isolated from a bronchitis vaccine that is being marketed in the United States.

In the genetics area, isolates of Newcastle disease virus on initial recovery and after long term propagation in laboratory host systems consist of heterogenous populations, the components of which differ in plaque type and in many other identifiable characters. The segregated components can be maintained pure for plaque type for only a limited number of passages. The relationship of plaque type to other characters, the degree of phenotyphic variation, and the rate of mutation are subject of study.

A system for establishing degrees of serological relationship based on an index which uses kinetics of neutralization data appears feasible.

Studies in the third area revealed that the pathogenesis of Newcastle disease virus in chickens, following an aerosol exposure, is affected by environmental conditions (air pollutants) and by the immune status of the individual. Only aerosols of a size that penetrate the entire respiratory system appear to induce solid immunity.

In the area of epizootiology, neutralizing (titers of 1,000 to 100,000) and hemagglutinin-inhibiting (titers of 40-320) substances for Newcastle disease virus were found in a high percentage of both the juvenile and adult members of a migratory bird population. Adults will die if massive doses of virus are given. Lesser doses that would be lethal to chickens are tolerated and the birds develop neutralizing antibody. Susceptibility of juveniles and persistence of antibodies in adults and juveniles is under investigation. On the basis of preliminary data, it appears that the lethality gradient of Newcastle disease virus strains for chickens is not true for this host. The relationship of Newcastle disease virus and this migratory bird will be carefully studied as the perpetuation of the infection in them is most readily understandable if one assumes a cycle of disease independent of the domestic fowl. (Wisconsin) (ADP a5-18)

F. Infectious Bronchitis

At the National Animal Disease Laboratory, Ames, Iowa, basic studies on the causative agent of infectious bronchitis are being conducted. A comparison of the embryonating egg, chicken embryo kidney (CEK), and chicken embryo liver (CELi) cell cultures revealed the egg to be 12-40 times more sensitive to infectious bronchitis virus (IBV) than CEK cells and about 500-1000 times more sensitive than CELi cells. Plaque counts in both CEK and CELi cells followed a linear relationship with virus concentration, indicating that one virus particle initiates a plaque. Fifty percent cell culture infective

dose titers in kidney and liver cell cultures were equivalent to plaque titers showing that all cell culture infecting virus was also plaque forming virus. There were no differences in adsorption of virus to both cell types. One step growth curves in liver cells show that the eclipse phase was longer and virus production and release was much slower than in kidney cells. Virus particles could not be selected nor altered by adaptation to increase the plaquing efficiency on the different cell types.
(NADL) (ADP a5-23)

G. Avian Encephalomyelitis

At the National Animal Disease Laboratory, Ames, Iowa, recent field studies of avian encephalomyelitis have provoked the question of marginal vitamin E deficiency as a factor in the development of this viral disease. The studies described herein are an effort to determine this possibility.

Preliminary experiments, using semisynthetic vitamin E deficient diets, were conducted to determine the type and severity of lesions as well as the time of their occurrence. Syndromes of encephalomalacia, exudative diathesis, and muscular dystrophy were produced in day-old chicks placed on a torula yeast base diet. Changes in the per cent of lipid were responsible for these variations in the disease process. Nervous system lesions occurred only in chicks on 9 per cent dietary lipid. The muscle lesions were shown to be due to direct damage to mitochondria.

The dietary regimen producing neural lesions was to investigate the effects of avian encephalomyelitis virus in vitamin E deficient chicks. It was shown that vitamin E deficient chicks do not develop as severe an infection as non-deficient chicks. It is doubtful, therefore, if vitamin E deficiency would be a predisposing factor in the development of avian encephalomyelitis. (NADL) (ADP a5-27)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Ornithosis

Bates, H. A., Pomeroy, B. S., and Reynolds, D. P. 1965. Ornithosis: Immunization of Turkeys with Miyagawanella of low virulence. Avian Diseases 9:220-226.

Bates, H. A., Pomeroy, B. S., Seal, U. S., and Jay, A. R. 1965. Ornithosis: Experimental Immunofluorescent Studies. Avian Diseases 9:24-30.

Beasly, J. N., and Walkins, J. R. 1965. Ornithosis in Chickens: Experimental Infection with a Virulent Turkey Strain of the Agent. Southwestern Vet., 18:2:199-202.

Kinjo, T., and Bankowski, R. A. 1965. Properties of Complement-fixation antigens prepared from the 6B6 ornithosis agent and Herellea-like (HL) bacteria with immune serums. Avian Diseases 9:3:359-367.

Livingston, C. W., Jr., Moore, R. W., Redmond, H. E., and Hardy, W. T. 1965. Polyarthritis Virus - A Cause of "Stiff Lambs" in Texas. Southwestern Vet., 18:4:279-281.

Pierce, K. R., and Moore, R. W. 1965. Meningoencephalitis in Turkeys Experimentally Infected with Ornithosis. Avian Diseases 9:2:266-272.

Chronic Respiratory Disease Complex

Anderson, D. P., Cherms, F. L., and Hanson, R. P. 1964. Studies on measuring the environment of turkeys raised in confinement. Poult. Sci., 43:305-318.

Anderson, D. P., Beard, C. W., and Hanson, R. P. 1964. The adverse effects of ammonia on chickens including resistance to infection with Newcastle disease virus. Avian Diseases 8:369-379.

Anderson, D. P., and Hanson, R. P. 1964. The influence of the environment on viral diseases of poultry. Presented at the 101st Ann. Meet. AVMA, Chicago, July 19-23. Avian Diseases 9:171-182.

Chute, H. L., Stauffer, D. R., and O'Meara, D. C. 1964. The Production of Specific Pathogen Free Broilers in Maine. Agr. Exp. Sta. Bull. 633.

Chute, H. L., Stauffer, D. R., Cuozzo, R. F., and O'Meara, D. C. 1964. Progress in PPLO eradication in chickens in Maine. Maine Farm Research July.

Chute, H. L., Cuozzo, R., Stauffer, D. R., and MacDonald, Vaughn. 1965. The Commercial Production of PPLO Free Chickens. Can. Vet. Jour., Jan. pp. 16-21.

Chute, H. L. 1964. Studies on the Commercial Production of SPF Poultry. Pres. USLSA Meet. Memphis, Tenn., Oct. 17-21.

Domermuth, C. H., Nielson, M., Freundt, E. A., and Birch-Anderson, A. 1964. Ultrastructure of Mycoplasma species. Jour. of Bact., 88:727-744.

Domermuth, C. H., Nielson, M., Freundt, E. A., and Birch-Anderson, A. 1964. Gross morphology and ultrastructure of Mycoplasma gallisepticum. Jour. of Bact., 88:1428-1432.

Dutta, S. K., Dierks, R. E., and Pomeroy, B. S. 1965. Electron microscopic studies of the morphology and stages of development of Mycoplasma gallisepticum. Avian Diseases 9:241-251.

Fabricant, J., and Boyer, C. I. Jr. 1964. Mycoplasma other than M. gallisepticum associated with airsacculitis in turkeys. Proc. 36th N.E. Conf. on Avian Diseases, North Carolina State University, Raleigh. June.

Gross, W. B. 1964. Retained caseous yolk sacs caused by Escherichia coli. Avian Diseases 8:438-441.

Hall, C. F., Flowers, A. I., Grumbles, L. C., and Moore, R. W. 1965. Turkey Condemnation Causes. Technical Report No. 1, Texas Agri. Exp. Sta.

Olesiuk, O. M., Van Roekel, H., and Chandiramani, N. K. 1965. Control of experimental Mycoplasma gallisepticum infection in young chickens with tylosin and other antibiotics. Avian Diseases 9:67-77.

Patterson, W. C. 1965. Recent Research on the Chronic Respiratory Disease Complex. Presented at Dedication of South Central Poultry Research Laboratory, State College, Mississippi, May 28.

Patterson, W. C. 1965. Environmental Research Related to Poultry Health Problems. Presented at 58th Ann. Meet. American Soc. of Agricultural Engineers, Athens, Georgia, June 22.

Payne, J. B. 1965. The Role of Quail Bronchitis Virus as a Possible Precipitating Factor in "Air Sac Syndrome" of Chickens. Thesis, Texas A&M University.

Sabry, Mohy, and Fabricant, Julius. 1965. An egg yolk overlay medium for the cultivation of avian mycoplasma. Proc. 37th N. E. Conf. on Avian Diseases, University Park, Pennsylvania, June.

Vardaman, T. H. 1965. Field Experiences on Mycoplasma gallisepticum-free Chickens. Pres. Georgia Veterinary Medical Assn. Annual Meeting, Jekyll Island, Georgia, June 19.

Salmonellosis

Williams, J. E. 1964. Salmonellosis - A Problem Challenging both Understanding and Action. American Poultry and Hatchery Federation News, August-September.

Williams, J. E. 1964. Paratyphoid and Arizona Control in Poultry. Pres. Poultry Management School, Mississippi State University, State College. September.

Williams, J. E. 1965. A Technique for Sampling Microbial Penetration Through the Shell and Shell Membranes of Chicken Eggs. Presented Southern Conf. on Avian Diseases, Gainesville, Florida. April.

Williams, J. E. 1965. Salmonella Control in Poultry Flocks. Pres. Southeastern Poultry and Egg Conference, Atlanta, Georgia. January.

Williams, J.E. 1965. Paratyphoid Control in Poultry. Presented "Broiler Day," University of Georgia, Athens. May.

Pasteurellosis

Heddleston, K. L., and Watko, L. P. 1965. Fowl Cholera: Comparison of Serologic and Immunogenic Responses of Chickens and Turkeys. Avian Diseases 9:3:367-376.

Heddleston, K. L., Rebers, P. A., and Ritchie, A. E. 1965. Immunizing and Toxic Properties of Particulate Antigens from Two Immunogenic Types of Pasteurella multocida of Avian Origin. Federation Proc. 24(2):3149.

Infectious Bronchitis

Lukert, P. D. 1965. Comparative sensitivities of embryonating chicken's eggs and primary chicken embryo kidney and liver cell cultures to infectious bronchitis virus. Avian Diseases 9:2:308-316.

AREA NO. 6 - INFECTIOUS AND NON-INFECTIOUS DISEASES OF FUR ANIMALS INCLUDING RABBITS

Problem. In the raising of fur animals in captivity, such as rabbits, chinchillas, mink, and foxes, disease problems incidental to the confinement of such animals are encountered. These include viral, bacterial, parasitic, mycotic, nutritional, and hereditary diseases. The enteric disease-complex causes great mortality in commercial rabbit production. It destroys whole litters and commonly attacks all susceptible rabbits on a farm. The respiratory disease-complex, perhaps, is second as a cause of mortality. In severe outbreaks over 50 percent of adult animals may die. These two diseases cause great economic loss to the rabbit industry, which produces an estimated 50 million pounds of meat annually and millions of dollars worth of rabbits for experimental purposes. Virus diseases of mink cause the greatest loss to the 7,000 mink ranchers now producing more than 5 million pelts annually valued in excess of $100 million. The role of helminths as carriers of rickettsial and viral agents causing, or associated with diseases of fur animals, is becoming of extreme importance and is one about which little is known.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving microbiologists and veterinarians engaged in both basic studies and the application of known principles to the solution of infectious and non-infectious diseases of fur animals. Research was conducted on the following diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 3 professional man-years. This effort was applied as follows:

Rabbit diseases 1.0 at the U. S. Rabbit Experiment Station, Fontana, California, in cooperation with the Animal Husbandry Research Division, ARS.

Coordinated Field and Laboratory Studies 1.0 at the U. S. Fur Animal Disease Research Laboratory, Pullman, Washington, in cooperation with the Washington State University.

Transmission of Infectious Diseases by Helminths 1.0 at the U. S. Fur Animal Disease Research Laboratory, Pullman, Washington, in cooperation with the Washington State University.

PROGRAM OF STATE EXPERIMENT STATIONS

Scientists at several of the State experiment stations are conducting research on the prevention and control of diseases of fur-bearing animals.

Investigators seek to determine the cause and control of Aleutian disease in mink. Basic studies are being made concerning the transmission of the disease. The relationship of nutrition, heredity and toxins as possible causative factors is being determined. Additional studies seek to identify the etiology of enteritis in mink and to develop methods for prevention and control.

Workers also seek basic information concerning the importance and the disease-producing characteristics of pseudorabies virus in mink. Attempts are being made to provide useful information concerning the cause and control of a scrapie-like disease in mink.

A modified live-virus vaccine has been developed for the prevention of myxomatosis in rabbits. Attention is now being directed toward evaluating the vaccine and determining its effectiveness against possible other strains of the virus.

The total State scientific effort devoted to fur-bearing animal disease research is 5.7 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Rabbit Diseases

At the U. S. Rabbit Experiment Station, Fontana, California, a survey of normal symptom-free animals indicated that 70% harbored the Pasteurella organism and 16% harbored Bordetella organisms in the nasal turbinates. The feeding of aureomycin and sulfamethazine, singly and together, had varied effects on the incidence. Aureomycin medication at 100 grams per ton of feed greatly reduced the incidence of nasal Pasteurellosis in mature animals, but had little or no effect on the fryer-size animals. Aureomycin medication also reduced the incidence of Bordetella isolations in the fryer-size animals. When aureomycin and sulfamethazine were combined, a high incidence of gastro-enteritis was encountered. Because of this high incidence of enteritis and resulting poor production, the use of this combination of drugs is not indicated in rabbit feeds.

An amylase-containing feed additive was fed to a group of animals at 2 - 4 pounds per ton levels. The addition of amylase to the ration had no effect on the incidence of enteritis. Blood amylase determinations were made on 36-day, and 7-week old fryer-sized animals. The lack of amylase in the ration does not appear to be involved with the incidence of enteritis.

Serum agglutinins produced by three commercial vaccines were studied. Agglutination levels were the highest and persisted for the longest period of time in those animals given an oil-emulsified Pasteurella vaccine. (Fontana, California) (ADP a6-5 ADP a6-6)

B. Field and Laboratory Study of the Diseases of Fur Animals

The following work has been reported from the Division's Fur Animal Disease Research Laboratory, Pullman, Washington:

Feline Cell Culture Propagated Panleukopenia Virus. Conventional neutralization tests performed in feline kidney cell cultures, using the Philips Roxane (PR) strain, and various feline virus immune sera revealed that the PR strain is either panleukopenia or a virus closely related to it. These tests also confirmed the relationship between feline panleukopenia and mink virus enteritis since both viruses stimulated the production of antibody which neutralized the Philips Roxane virus.

An antigenic extinction test using the PR virus revealed that the efficacy of cell culture fluid as a vaccine was about 300 times greater than its infectivity when measured by titrations in feline kidney cell culture.

Intranuclear inclusions were produced in cell culture. There was a relationship between the percentage of inclusion bodies and virus titer. As the titer increased, the percentage of mature inclusions also increased.

Adjuvated-Inactivated Distemper Vaccine. There was no significant difference in the ability of live or adjuvated inactivated distemper vaccines to overcome the blocking effect of maternal antibody.

Some physical and Chemical Characteristics of Partially Purified Aleutian Disease Virus. The protein content of tissue preparations can be reduced by fluorocarbon extraction without detectable effect on the biological activity of the virus. Partially purified Aleutian Disease agent showed a surprising stability against the action of proteolytic enzymes and nucleases. Conversely, it is readily inactivated by boiling or treatment with strong acids, bases or iodine. (Pullman, Washington) ADP a6-7)

C. Transmission of Viral and Rickettsial Diseases of Helminths

At the Division's Fur Animal Disease Research Laboratory, Pullman, Washington, recent studies have demonstrated 5-1/4 years persistence of the Elokomin fluke fever agent in metacercariae of Nanophyetus salmincola recovered from steelhead trout maintained in salt water ponds. Juvenile silver salmon recovered off the coast of Alaska confirmed our previous studies demonstrating a high degree of infectivity of Pacific salmon with the vector fluke. Ten percent of these salmon, which were believed to be northbound migrants, were demonstrated to be infected with the metacercariae of

N. salmincola. A comparison of Elokomin fluke fever and the Sennetsu rickettsial agent of Japan is under way. (Pullman, Washington) (ADP a6-8)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Rabbit Diseases

Hagan, K. W. 1965. Rabbit Ailments and Treatments. Amer. Rabbit Breeders Assoc. Official Guidebook, Sect. 9, pp. 84-89.

Diseases of Fur Animals

Burger, D., and Gorham, J. R. 1964. Response of Ferrets and Mink to Vaccination with Chicken Embryo-Adapted Distemper Virus. Archiv. für die Gesamte Virusforschung, 14:449-461.

Burger, D., and Gorham, J. R. 1964. The Duration of Immunity Following Vaccination with Chicken Embryo-Attenuated Vaccine. National Fur News, 36:29.

Burger, D., and Gorham, J. R. 1964. The Onset of Distemper Immunity Following Modified Vaccine. National Fur News, 36:26.

Gorham, J. R. 1964. Toxic Food Poisoning of Unknown Origin. National Fur News, 36:18.

Gorham, J. R. 1965. Growing Viruses in the Laboratory. National Fur News, 36:20.

Gorham, J. R. 1964. Living Virus Vaccines. Washington State Review, Washington State University.

Gorham, J. R. 1964. Mink Virus Enteritis. National Fur News, 36:11.

Gorham, J. R. 1964. Foreign Bodies. National Fur News, 36:11.

Gorham, J. R. 1964. An Attempt to Immunize Against Distemper: Newborn and Unborn Kits. National Fur News, 36:18.

Lo, J. P., Dickson, W. M., and Gorham, J. R. 1964. Errors in Distemper Virus Titrations Performed in Embryonated Eggs. Archiv. für die Gesampte Virusforschung, 15:74-90.

Ott, R. L., Gorham, J. R., and Farrell, R. K. 1964. Note on the Use of Antiserum and Live Distemper Virus Vaccination. Aust. Vet. Jour., 40:267-268.

Padgett, G. A., Reiquam, C. W., Leader, R. W., and Gorham, J. R. 1965. P.A.S. Positive Material Deposited in the R.E. System and Neurons of Man and Animals with the Chediak-Higashi Syndrome. Fed. Proc.

AREA NO. 7 - MISCELLANEOUS INFECTIOUS AND NON-INFECTIOUS DISEASES OF ANIMALS

Problem. Included in this area of research are studies on problems involving more than one species of domestic animal, poisoning by various plants, which differ in toxicity according to local conditions, and affect different species of animals in various ways; agricultural chemicals such as herbicides and pesticides, which may produce poisoning in animals, especially if not properly used, and may also leave dangerous residues in the soil, feed, or animal body, and bloat, a common, serious condition in cattle and sheep. Investigations of these diverse problems require modern techniques as well as fundamental approaches through chemistry, pathology, physics, physiology, and other scientific disciplines. The problems are so complex, diverse, and numerous that it has been impossible to more than scratch the surface in probing for basic knowledge required for protection of the nation's livestock and poultry populations.

USDA AND COOPERATIVE PROGRAM

The Department has a continuous long-term program involving biochemists, microbiologists, pathologists, physicists, and veterinarians engaged in both basic studies and the application of known principles to the solution of miscellaneous infectious and non-infectious diseases of animals. Research is being conducted at the designated locations.

The Federal scientific effort devoted to research in this area totals 21.4 professional man-years. This effort is divided among sub-headings as follows:

Components of Normal and Immune Serum 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Bloat in Ruminants 3.3 at the National Animal Disease Laboratory, Ames, Iowa, and through cooperative agreements with the California, Maryland, Mississippi, and Wisconsin Agricultural Experiment Stations, and with the New York State Veterinary College.

Preparedness for Diagnosis of Foreign Animal Diseases 0.5 at the Plum Island Animal Disease Laboratory, Greenport, Long Island, New York.

Toxicology and Pathology Related to Insecticides 3.0 at the ADP Field Station, Toxicology Laboratory, Kerrville, Texas, in cooperation with the Entomology Research Division.

Biochemical Effects of Agricultural Chemicals 1.0 at the ADP Field Station, Toxicology Laboratory, Kerrville, Texas, and through a cooperative agreement with the Stephen F. Austin College at Nacogdoches, Texas.

Detoxication Mechanisms in Cattle and Sheep 1.0 at the ADP Field Station, Toxicology Laboratory, Kerrville, Texas.

Cytological Responses to Antiparasitic and Other Agricultural Chemicals 1.0 at the ADP Field Station, Toxicology Laboratory, Kerrville, Texas.

Poisoning by Plants 1.1 at the Logan, Utah, Field Station, through formal cooperation with the Utah Agricultural Experiment Station, and informal cooperation with the U. S. Plant, Soil and Nutrition Laboratory of the Soil and Water Conservation Service, Ithaca, New York. A PL 480 grant supports research at the Instituto Biologico, Sao Paulo, Brazil, on The Study of Plants of the State of Sao Paulo Poisonous to Domestic Animals.

Toxicity of Herbicides and Herbicide-Treated Plants for Domestic Animals 0.5 at the Logan, Utah, field station, with informal cooperation with the Utah Agricultural Experiment Station and the Crops Protection Branch of the Crops Research Division at Logan, Utah.

Biological Changes Associated with Neuropathological Conditions in Animals 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Physiopathological Investigations of the Interrelations between the Respiratory, Circulatory, and Digestive Systems of Animals 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

Proteins and Other Complex Molecules from Animal Disease Agents Derived Primarily from Surface Structures and Extracellular Products 1.5 at the National Animal Disease Laboratory, Ames, Iowa.

Chemical and Physical Studies on Microbial Antigens 1.5 at the National Animal Disease Laboratory, Ames, Iowa.

Microbiology of the Ruminant Digestive Tract and Its Relation to Digestive Disturbances 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

PROGRAM OF STATE EXPERIMENT STATIONS

There are numerous complex problems in this area which need attention. Increased emphasis is being placed on research aimed at the toxicological and pathological effects associated with the use of insecticides and other agricultural chemicals. Efforts are being made to develop and evaluate more specific and less persistent chemicals for the control of livestock diseases. Also, considerable attention is directed toward non-chemical control measures.

A number of States are identifying and studying the pharmacological action of the toxic principals involved in major poisonous plants. Treatment measures are being continuously developed and evaluated. Other research is aimed at the toxicology and metabolic fate in animals of nitrates, urea, selenium, fluorides, lead, molybdenum, etc.

Work continues at a number of locations on the cause and prevention of bloat in ruminants. Investigations are being made concerning plant and animal interactions which contribute to the bloat problem. Preventive and treatment measures are being developed and evaluated at the same time.

Other problems and conditions receiving attention by State research scientists are various types of livestock tumors, hypertension, anethesia, improvement in slaughter methods, neurophysiology, function studies on the hypothalmus, thyroid and adrenal glands, syndactylism, bone growth, hemodynamics and numerous physiological, neurological and anatomical studies.

The total research effort devoted to miscellaneous diseases of animals at the States is 32.7 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Components of Normal and Immune Serums.

At the National Animal Disease Laboratory, Ames, Iowa, changes in serum proteins were studied by paper electrophoresis for 29 weeks following exposure in midpregnancy of 18 Brucella abortus strain 19 vaccinated, and 5 nonvaccinated heifers to virulent Br. abortus strain 2308. In the serums of heifers that became infected (4 vaccinated and 4 nonvaccinated), the relative percentage of gamma globulin increased, whereas that of albumin decreased. The percentage of gamma globulin finally exceeded that of albumin. The excess of gamma globulin over albumin was greater and persisted longer in serums from infected nonvaccinated than in infected vaccinated heifers. The changes in the amount of gamma globulin roughly paralleled the changes in the serum agglutinin titers. Only minor changes occurred in the concentrations of albumin and gamma globulin in the serums from 14 vaccinated and 1 nonvaccinated heifer that did not become infected.

(NADL) (ADP a7-14)

B. Bloat in Ruminants

Under a cooperative agreement with the Maryland Agricultural Experiment Station, College Park, studies designed to identify the active factor(s) in green legumes which dispose to bloat have been conducted. Alcohol extracts, saline extracts, alcohol and water extracts, and saline soluble protein have been prepared from green alfalfa. None of these preparations has given any consistent results in producing bloat in fasted sheep, in sheep fed a high concentrate bloat diet, or in sheep previously grazed on grass or ladino clover. In trials conducted during the past year, injections of atropine and adrenaline have consistently produced slight to moderate bloat in sheep on a variety of diets, and tolazoline hydrochloride and reserpine have been effective in diminishing bloat so produced.

Tyramine and/or tyrosine, administered orally to sheep injected with atropine, have not produced bloat as frequently as has the injection of epinephrine. Attempts to demonstrate atropine-adrenaline-like action in the preparations from green alfalfa have not been successful and the incidence of bloat in sheep grazed on or fed the alfalfa was quite low.
(College Park, Maryland) (ADP a7-15)

At the New York State Veterinary College, Ithaca, under a cooperative agreement, research has revealed that the transport of a fatty acid from the rumen contents to blood depends upon 1) the electrochemical gradient of the anion form, 2) the chemical gradient of the undissounted acid form, 3) the relative permeability of the epithelium to the anion and acid forms, and 4) the rate at which the fatty acid is metabolized by the epithelial tissue. Therefore, the isolated, short-circuited rumen epithelium was used to study the effects of pH, concentration gradient, imidazole vs. bicarbonate buffer systems and fatty acid metabolism on the transport of acetate propionate and butyrate. Transport was found to increase with an increase in concentration gradient or a decrease in the pH of the fatty acid solution bathing the lumen surface of the tissue, and acetate transport also was greater when the bathing solutions were buffered with bicarbonate in place of imidazole. The effect of bicarbonate buffer on acetate transport was associated with a change in short-circuit current which suggested the HCO_3 or CO_2 also affected the active transport of ions. All three fatty acids were metabolized to a considerable extent by the tissue but their metabolism was not a simple function of the amount of fatty acid absorbed. The results, including the effect of anoxia on transport, indicated that metabolism of each of the fatty acids played a critical role in determining its rate of absorption and transport.
(Ithaca, New York) (ADP a7-15)

Researchers at the Wisconsin Agricultural Experiment Station, Madison, under a cooperative agreement, report that, in order to understand better the processes of digestion before and during the onset of bloat, experiments were carried out to determine those enzymatic activities or reactions which, by their qualitative or quantitative behavior, may influence those pathways leading to increases in the viscosity of ruminal contents. Bloat-regulating substances operating in or administered to ruminants, were also studied.

To determine which ions produced pectate gels like that of calcium, cations of zinc, aluminum, iron (ferric), copper, potassium, magnesium, manganese, and sodium were tested. Of the cations used only potassium, magnesium (sulfate), and sodium failed to form some type of gel; the other salts formed a lumpy or semi-gel. Only calcium salts formed stable pectate gels and therefore served as good models for producing a viscous rumen during bloat.

Properties of pectin methyl esterase (PME) activity in a commercial pectin esterase and in alfalfa extracts were examined and the optimal pH's, substrate and enzyme concentrations, and time of reaction were found by

titration of pectin. In addition, PME activity was found to be more inhibited at lower concentrations of the biodegradable detergent, ultrawet K soft, than by the nonbiodegradable one, K dense. The K soft surfactant has not yet been used in feeding trials of cattle during experimental bloat.

PME activity and moisture content of growing alfalfa were greater on bloating than on non-bloating days. Freezing enhanced the extraction of PME. Ruminal contents were a better PME extractor than buffered saline.

The hydroxamic acid reaction (HAR) for the presence of pectin in agar medium has been used to measure PME activity since the clear zones represented sections on the plate where the enzyme has hydrolyzed the methyl ester groups of pectin. Salts in general had very little effect on the size of clear zones but added phosphate buffer produced slightly larger zones than other salts added to acetate. The relative PME in ruminal fluids was about 0.013 percent. Larger concentrations of detergent up to 0.10% did not reduce the apparent PME activity in strained ruminal contents. The HAR method has been used successfully for a large number of experiments.

It has been shown that cellulase activity was measured by the carboxymethyl cellulose-agar pectin dish technique; cellulase was affected by pH of the substrate, time and temperature of incubation, and concentration of the carboxymethyl cellulose (CMC) in the substrate-agar system. Lower pH's, longer periods of incubation, higher temperatures and lower concentrations of CMC gave larger areas of activity. Alkyl aryl sulfonate did not inhibit cellulase activity.

In further studies with starch-degrading enzymes, it was shown primarily that centrifuged ruminal fluid from a cow bloated on pure corn and alfalfa contained 100 times more starch enzyme activity than that of non-bloated ruminal fluids.

Frothy digesta erupted from a cow resembled that of "feed-lot" bloat. The viscous nature, low pH and the yellow particles of corn entrapped in the stable foam were characteristic properties of corn or grain bloating. A large increase in starch-degrading activity and smaller increases in PME and protein-degrading activities all complement the increased viscid ruminal contents of the bloated cow. (Madison, Wisconsin) (ADP a7-15)

C. Toxicological and Pathological Effects of Insecticides, Herbicides, Fungicides, and other Agricultural Chemicals on Livestock and Poultry.

At the Division's Toxicology Laboratory, Kerrville, Texas, the following work is reported:

During FY 1965, 72 insecticides were studied in cattle, sheep, and goats, many of them as cooperative ventures with the Entomology Research Division. As in past years, the toxicities were found to run from the impossibly dangerous to reasonable safety. Of particular interest as compounds of low toxicity are ENT 25841 produced by Shell Chemical as compound 8447, and

ENT 27162 produced by Cela of Germany and sometimes called Bromophos.

Seven insecticides were studied in 280 chickens 8 to 9 weeks of age to determine the oral toxicity. The maximum nontoxic dosages found were: coumaphos, 2.5 mg./kg.; diazinon and dichlorvos, 5 mg./kg.; Ciodrin and ruelene, 100 mg./kg.; and dioxathion, 250 mg./kg.

Studies were begun in the last half of FY 1965 of insecticides employed primarily as plant treatments. Past capability permitted studies of those used directly on livestock but did not allow us to glean more than a small amount of knowledge of those used on plants. Initial studies have been with sheep to establish techniques.

One of the more interesting findings at this time is that sheep do not consume demeton (Systox), even when partially starved, when it is added to feed or sprayed on grass. This finding needs to be enlarged upon and firmly established for both cattle and sheep. If true, then poisoning of sheep and cattle would be most unlikely in a pasture where both treated and untreated forages are available.

In one study sheep were observed to increase their tolerance to malathion as both the dosage and frequency of administration were increased.

Analysis of dips revealed the depletion of each compound from the dipping fluid while wool samples from sheep in the order of their immersion demonstrated the differences in the amounts of insecticide retained on each animal.

Co-ral and one approved lindane formulation were found to maintain the original concentration throughout dipping. One toxaphene formulation was found to deplete slowly. Most other compounds and formulations depleted rapidly.

When depletion was small or nonexistent the wools of sheep received rather uniform deposits. When depletion was marked the wool deposits were highly variable.

Apholate was fed to Jersey cattle at 1 mg./kg. throughout one gestation period. A deficiency of white blood cells appeared in one heifer after 80 daily doses and in the others after 110. One heifer died after 335 doses, one delivered a calf and died after 531 doses, one delivered prematurely and survived 581 doses, and the fourth delivered at term and survived 629 doses.

Chronic feeding of apholate was continued in sheep that were also in study during FY 1964. Two rams and three ewes survived 638 daily doses at 1 mg./kg. A fourth ewe died after 574 daily doses. A moderate deficiency of white cells was developed and persisted for 128 days after the withdrawal of apholate.

Hempa appears to be of considerably less toxicity than apholate, tepa, and metepa, but shares with them the ability to produce a deficiency of white blood cells.

Twenty-one commercially available herbicides were administered orally to chickens, sheep, or cattle, in repeated daily doses. Virtually all proved to be of a low order of toxicity, indicating that the hazards of most of these compounds to domestic animals would be limited to cases of accidental spillage and similar incidents rather than the consumption of them on treated forage or feed. The study established the clinical signs and necropsy lesions to be expected in these poisonings.

Captan, Zineb and Ceresan M were studied in chickens or cattle; of the three Ceresan M is the more toxic. Cattle appear to be from two to three times more susceptible to poisoning by Ceresan M than are chickens. Massive doses of Zineb or Captan were required to produce poisoning.

(Kerrville, Texas) (ADP a7-23)

D. Biochemical Effects of Agricultural Chemicals and Control Substances

The following studies were reported from researchers at the Division's Toxicology Laboratory, Kerrville, Texas.

Injured or dying cells are known to leak, or discharge, enzymes into the blood stream. Certain enzymes are to be found extensively only in specific tissues such as the heart, muscles, or liver. Research during FY 1965 was devoted to determining patterns of these enzymes in average cattle then, by poisoning cattle, attempting to develop a "fingerprint" pattern of the enzymes that increased. In addition, studies were made of the effect of oxime-type cholinesterase reactivators on this "fingerprint" pattern. Results have, in general, been encouraging. The oximes appear to protect many cells from injury in addition to their key action of being antidotes for organophosphorus insecticide intoxication.

Dioxathion (Deltox). Cattle were poisoned with dioxathion to determine the effect upon certain serum enzyme systems and blood protein elements. Three oximes, 2-PAM, DAM, and TMB_4, were used as antidotes at 10 and 20 mg./kg. Cattle poisoned by dioxathion show elevations in the activity of serum glutamic oxalate and pyruvate transminases, and of alkaline phosphatase, which is indicative of tissue damage. Blood beta lipoprotein levels were elevated, while gamma globulin was decreased. 2-PAM and TMB_4, when administered to cattle poisoned with dioxathion, retain the levels of the serum glutamic oxalacetic transaminase, glutamic pyruvic transaminase, and alkaline phosphatase activities at near normal. 2-PAM, at both dosage levels, lowered the level of beta lipoprotein of poisoned animals while only the higher dosage of 20 mg./kg. of TMB_4 did so. With all three oximes the level of gamma globulin of poisoned animals remained near normal. DAM did

not aid in reducing fatalities. At the dosages studied TMB_4 appears to be slightly more beneficial than 2-PAM for dioxathon poisoned cattle insofar as biochemical effects are concerned. DAM does not appear to be beneficial at the levels studied.

Dichlorvos (Vapona). Cattle were poisoned with an oral dosage of dichlorvos to determine the effects on serum glutamic oxalacetic and pyruvic transaminase, aldolase and alkaline phosphatase. Oximes were given to some of the cattle to determine their protection of those enzyme systems. Severe poisoning of cattle did not follow the dosages of dichlorvos that were administered. 2-PAM and DAM retained the enzyme activities of the mildly poisoned animals near normal, whereas TMB_4 appeared to cause an increase in activity above normal during the test. From the biochemical standpoint, it appears that 2-PAM and DAM offer more protection to the enzyme activities than does TMB_4.

Coumaphos (Co-ral). Cattle were poisoned with coumaphos and enzyme systems studied in serum. Some of the cattle received antidotal therapy with 2-PAM. Glutamic dehydrogenase, sorbital dehydrogenase, phosphohexose isomerase and serum arginase were studied in an effort to find significant enzyme activity alterations indicative of possible tissue change. No significant differences were noticed in the enzyme activities regardless of treatment. 2-PAM reduced mortality in coumaphos poisoned animals, but this benefit could not be detected in the enzyme studies. (Kerrville, Texas)

In cooperative work at the Stephen F. Austin State College, Nacogdoches, Texas, the problem of scattered sound intensity from a rigid sphere was programed on the IBM 1620 computer. This showed that calculation errors were previously made in working out this problem. However, the theoretical and observed scattering pattern are still not in agreement.

The relationship of aerosol drop size to the change of sound velocity at various frequencies has been re-done with less experimental error and greatly improved techniques. As yet final calculations on drop sizes determined by this method are not available.

A modification of the Ionovac Speaker has shown that the greatest sound intensity can be obtained from the speaker if it is excited with 23 megacycles RF rather than the design frequency of 27 megacycles. Also the RF excitation does not cause air to ionize but does cause excitation of the nitrogen in the air and thus emission of light.

In other research at this location, solubility studies on potassium antimony tartrate were made at several temperatures. Also the solubility of barium chloride in saturated solutions of potassium antimony tartrate was measured at several temperatures. Apparently two solid phases can exist in this system. Investigation of the composition and properties of each is being continued. (Nacogdoches, Texas) (ADP a7-18)

E. Detoxication Mechanisms in Cattle and Sheep

In studies at the Division's Toxicology Laboratory, Kerrville, Texas, certain oximes have shown an ability to reverse the cholinesterase inhibition induced by organic phosphorus compounds. The ability varies between oximes and between the compounds producing the inhibition.

During this year 2-PAM, DAM, and TMB_4 were the three oximes chosen for study. 2-PAM became available commercially this year. Each of the compounds was useful, but 2-PAM and TMB_4 appeared to be superior to DAM. Particularly encouraging was the beneficial effect of these compounds in cattle poisoned by coumaphos. Usually such animals do not readily respond to atropine, the pharmacologic antidote. Oximes combined with atropine markedly increased the number and speed of recoveries.

In cooperation with the Entomology Research Division, studies of residues of compound 4072 in cattle tissues were made. Spraying cattle with 0.1% emulsions at weekly intervals for 12 applications and at 2-week intervals for 6 applications produced small residues which disappeared within 28 days after the spraying was suspended.

A technique for the analyses of tissue residues of the herbicides 2,4,5-T, propylene glycol butyl ether ester and 2,4,5-T acid has been developed and applied in a test in which three sheep were poisoned by the herbicides. With both compounds residues were deposited primarily as the acid or its salt.

Residues deposited as the acid ranged from an average of 44 ppm in omental fat to 261 ppm in kidney with 73 ppm in muscle and 67 ppm in liver. Some residues were deposited as the ester. The highest was 1.25 ppm in kidney.

The above residues are in animals killed by massive dosing with 2,4,5-T and do not in any way represent the levels which would result from normal exposures. Such studies are planned for fiscal year 1966.

In cooperation with the Entomology Research Division, a number of water samples representing well and surface supplies in the vicinity of the Kerrville Laboratories, were analyzed for chlorinated pesticides. None were found. Sensitivity of the method used would have detected 1 part per billion or less. (Kerrville, Texas) (ADP a7-19)

F. Characterization of Cytological Response to Toxic Actions of Anti-parasitic and Other Agricultural Chemicals in Cattle and Sheep

At the Division's Toxicology Laboratory, Kerrville, Texas, research studies indicated that the polyfunctional alkylating agents apholate, tepa, and metepa, which are insect chemosterilants, injected into the yolk of fertile eggs just prior to incubation and into the yolk sacs of developing embryos after varying periods of incubation, induced congenital abnormalities in embryos that survived to the 18th day of incubation.

Doses of each compound at 250 µg. and higher per egg were lethal to 4-day-old embryos. Death usually occurred within 72 hours. Each compound at 125 µg. per egg was lethal to 1- and 2-day-old embryos in 72 - 96 hours, but allowed 4-day-old embryos to continue to develop for as long as 11 days before they died. Doses of each compound at 5.4 to 25 µg. per egg usually permitted embryos to develop. Each compound induced similar congenital abnormalities such as defects of the beak, eyes, digits, and legs; cerebral and visceral hernia; edema; growth retardation and reduced weight.

(Kerrville, Texas) (ADP a7-20)

G. Livestock Poisoning by Plants

Researchers at the Division's Poisonous Plants Laboratory at Logan, Utah, report on their work as follows:

Cyclopian-Type Malformation in Lambs. The teratogenic material of the plant Veratrum californicum, responsible for ovine fetal cyclopsia, has been subjected to extensive purification. Over 40 malformations and fetal deaths were produced with extracts and purified materials. A number of glycosides and parent alkamine steroidal alkaloids were isolated by fractional crystallization. Preliminary studies indicated that two of the glycosides and two of the parent alkamines were capable of producing the teratogenic effect. Infrared studies have allowed a limited correlation of the structural requirements for teratogenicity. (ADP a7-7)

(Logan, Utah and Ames, Iowa)

Physiopathologic Aspects of Lupinus sericeus and L. caudatus (crooked calf syndrome). The crooked calf syndrome continues to be a wide-spread problem throughout the Western States and Alaska. This disease entity has not been characterized and is often confused with other deformities such as bovine achondroplasia, arthrogryposis, internal and external hydrocephalus, prognathia, anophthalmia, cerebellar ataxia, contracture of tendons and spastic lethals.

The degree of skeletal-musculo deformities varies greatly in individual cases. If the malalighment and malpositioning of the limbs and vertebrae is not too severe, they appear to become apparently normal clinically as the animal matures. If the deformity is too severe at birth, then the malformation is exaggerated and becomes progressively worse as the animal matures.

This disease syndrome is very complex and evaluation of the lesions can be justly open to more than one interpretation. The musculo-skeletal system is structurally and functionally a unit, it can be fully understood only if this concept is kept in mind while artificially separated parts of it are studied.

Feeding of Lupine plant to pregnant Hereford heifers from the 22nd to 120th days of gestation caused congenital deformities and abortion. The malformed calves were characteristic of clinical field cases of the crooked calf syndrome.

The crooked calf syndrome has a wide spectrum of lesions. There appears to be some inhibition, arrest, or interference with the normal sequential differentiation and specialization of cells, tissues, and organs at a specific time in embryonic and fetal development. The spectrum of lesions indicates the time of insult may occur at different stages of embryogenesis. Limb malalignment and vertebral aplasia are characteristic of this disease entity. Anophthalmia and cyclopia have been seen in calves with skeletal lesions typical of this syndrome. Flexure of pastern joints can be differentiated from this condition by clinical history, signs and x-ray evaluations.

Definite management practices can be correlated with the crooked calf syndrome. Cows grazing irrigated and crested-wheat grass pastures all had normal calves, while cows grazing in Newman Canyon had a number of crooked-legged calves. Lupine taken from this range area was used to experimentally produce a congenital malformed calf typical of clinical field cases. There was no correlation between bulls used and the incidence of deformed calves. All abnormal calves were born during a definite short period of time in the early part of the normal calving season.

Numerous uncontrollable factors are present on range areas where the crooked calf syndrome is a problem. Seasonal variation of such factors could influence the seasonal incidence of the disease.

Based on information and data accumulated to date, the crooked calf syndrome appears to be non-hereditary in nature, however, associated with the ingestion of some toxic substance and/or substances during some stage of embryonic or fetal development.

If the cause and/or causes and lesions associated with the crooked calf syndrome can be more fully understood, it will be invaluable in a number of ways. It will save livestockmen large sums of money and permit wiser utilization of range areas. (Logan, Utah) (ADP al-28)

H. Studies to Develop Alleviators and Diagnostic Tests for Plant Poisoning, and Methods to Avoid Harmful Residues in Animal Tissues from Ingesting Chemically Treated Plants.

Researchers at the Division's Poisonous Plants Laboratory, Logan, Utah, report that feeding False Hellebore (Veratrum californicum) to ewes 7 and 21 days after being sprayed with 2-4D Ester and 2-4D Amine reduced the incidence but did not completely eliminate the plant's ability to cause congenital deformities when ingested by ewes on the 14th day of gestation.

The total amount of alkaloids in the veratrum plants, determined on dry weight basis, was not significantly decreased from the herbicidal treatments.

Further investigation will be made with other herbicides in cooperation with the Crops Research Division. (Logan, Utah) (ADP a7-17)

I. Mycotic Diseases of Domestic Animals

At the National Animal Disease Laboratory, Ames, Iowa, researchers on this subject report as follows:

Nocardiosis is an important infectious disease of cattle, dogs, and man. Systemic nocardiosis frequently mimics tuberculosis or systemic fungal infection and diagnosis is often delayed until the disease has reached terminal stages. An antigen has been developed and used successfully in skin tests, complement fixation tests, and agar gel precipitin tests of cattle experimentally infected with Nocardia asteroides. Methods for the standardized preparation and fractionation of the culture filtrate antigen were developed and the antigenic principal was found to reside in a single column fraction. This fraction was found to be active in immunologic tests for delayed cutaneous hypersensitivity, complement-fixing and precipitating humoral antibodies. Chemical analysis of the antigenic material indicated that its major constituents were protein (55%) and hexose (10%). Neither chemical nor immunologic evidence of cell wall constituents were detected - the latter evidence is of significance to the antigen's specificity. Further work to determine the range of specificity of this antigen is contemplated. (Ames, Iowa) (ADP a7-24)

J. Biological Changes Associated with Neuropathological Conditions in Animals

At the National Animal Disease Laboratory, Ames, Iowa, the mechanism of the production of hyperglycemia by Veratrum alkaloids has been studied in adrenalectomized sheep and in sheep treated with chemical adrenal blocking agents. Neither dihydroergotamine nor N-isopropyl methoxamine prevented hyperglycemia or other clinical effects of the alkaloid. In vitro studies with sheep tissue slices suggested that glucose metabolism was inhibited when tissues were incubated with the alkaloids. (Ames, Iowa) (ADP a7-26)

K. Physiological Investigations of the Interrelation between the Respiratory, Circulatory and Digestive Systems of Animals

At the National Animal Disease Laboratory, Ames, Iowa, work with the intravenous or intramuscular injections of ester alkaloid preparations from the plants Veratrum viride and V. californicum (0.2 to 10.0 mg/100 kg) resulted in vomition. Somewhat lower doses were effective in relief of non-frothy bloat in cattle within 5 to 10 minutes. However, the preparations were less effective in relief of frothy bloat. Doses effective in relieving bloat, enhancing rumination and stimulating vomition produced only minimal temporary muscular incoordination and hypotension which ceased within one hour.

Toxic factors chemically and physiologically similar to bacterial lipopolysaccharide endotoxin have been extracted from rumen bacteria and from cell-free rumen liquor. The similarities in physiologic response of sheep to intravenous administration of these materials with that of animals suffering from grain engorgement suggest that endotoxins from normal rumen microorganisms may play a significant role in the disease.
(Ames, Iowa) (ADP a7-27)

L. Proteins and Other Complex Molecules from Animal Disease Agents Derived Primarily from Surface Structures and Extracellular Products

Researchers at the National Animal Disease Laboratory, Ames, Iowa, found isolated flagella from two different biotype strains of Vibrio fetus to be antigenically identical and identical in chemical composition. Peptide maps, amino acid composition, N-terminal amino acid, and electrophoretic criteria were used to establish chemical compositional identity, while Ouchterlony gel diffusion was used to establish antigenic identity.

Two common soluble intracellular antigens from three bio-serotypically different Vibrio fetus strains have been extensively purified and are being subjected to chemical comparison. (Ames, Iowa) (ADP a7-28)

M. Chemical and Physical Studies on Microbial Antigens

At the National Animal Disease Laboratory, Ames, Iowa, studies have been completed on the characterization of several antigens. Formalinized saline extracts of cells of two strains of Pasteurella multocida of avian origin were fractionated by ultracentrifugation. A gel-like precipitate which had both toxic and immunizing properties for chickens and mice was obtained. A marked increase in the specificity of the serological reactions was obtained with the gel-like precipitate as compared to the unfractionated saline extract.

A nitrogen and carbohydrate-containing antigen has been isolated from the culture supernatant of Actinomyces bovis and purified by alcohol fractionation and column chromatography. (Ames, Iowa) (ADP a7-29)

N. Microbiology of the Ruminant Digestive Tract and its Relation to Digestive Disturbances

At the National Animal Disease Laboratory, Ames, Iowa, a new pathway for biosynthesis of phenylalanine has been demonstrated. Many pure cultures of rumen bacteria, the mixed ruminal population incubated in vitro, and also certain nonruminal anaerobic bacteria synthesize phenylalanine using the intact carbon skeleton of phenylacetic acid. Details of the biosynthetic mechanism are not known, but there is evidence suggesting a condensation of the carboxyl-carbon of phenylacetic acid with CO_2 to produce phenylpyruvic acid, which is then transaminated. (This is different from the previously described biosynthetic pathway shown in aerobic microorganisms

where the 3-carbon side chain of phenylalanine arises as a unit from an intermediate in glycolysis.) Limited data indicate that, in the rumen, tryptophan is synthesized from indoleacetic acid, presumably also by a carboxyl-carbon-CO_2 condensation reaction. (This is different from any known pathway of tryptophan biosynthesis.) These are apparently significant reactions and reflect microbial adaptation to the ruminal environment where phenylacetic and indoleacetic acid are usually present in appreciable quantities, while free phenylalanine and tryptophan are not.

Toxic factors chemically and physiologically similar to bacterial lipopolysaccharide endotoxin have been extracted from rumen bacteria and from cell-free rumen liquor. The similarities in physiologic response of sheep to intravenous administration of these materials with the physiology of animals suffering from grain engorgement suggest that endotoxins from normal rumen microorganisms may play a significant role in the disease. Intraruminal administration of endotoxin had little effect on sheep and conditions permitting absorption of intact endotoxin from the gastro-intestinal tract are not known. (Ames, Iowa) (ADP a7-30)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Components of Normal and Immune Sera

Rose, Joseph E., and Amerault, Thomas E. 1964. Electrophoretic and Ultracentrifugation Studies on Serum from Pregnant Heifers after Exposure to Virulent Brucella abortus. Amer. J. Vet. Res., 25(107):998-1001.

Bloat in Ruminants

Meyer, R. M., Bartley, E. E., Morrill, J. L., and Stewart, W. E. 1964. Salivation in Cattle I. Feed and Animal Factors Affecting Salivation and its Relation to Bloat. J. Dairy Sci., 47:1339-1345.

Meyer, R. M., Decker, J. L., Bartley, E. E., Stewart, W. E., Noordsy, J. L., and Stiles, D. A. 1965. Salivation Studies in Cattle with Bilateral Submaxillary and Parotid Gland Cannulas. J. Dairy Sci., 48:790.

Ryan, R. K. 1964. Concentrations of glucose and low-molecular-weight acids in the rumen of sheep following the addition of large amounts of wheat to the rumen. Amer. J. Vet. Res., 25:646-652.

Sellers, A. F., Stevens, C. E., Dodson, A., and McLeod, F. D. 1964. Arterial blood flow to the ruminant stomach. Amer. J. Physio., 207:371-7.

Stevens, C. E., and Stettler, B. K. 1965. Transport and metabolism of acetate, propionate and butyrate by bovine rumen epithelium. Fed. Proc. 24:276.

Stewart, W. E., and Henning, G. C. 1965. Esophageal-abomasal Anastomosis Procedure used to Study Forestomach Development. J. Dairy Sci., 48:17.

Toxicology and Pathology

Palmer, J. S. 1964. Toxicity of Methyluracil and Substituted Urea and Phenol Compounds to Sheep. Jour. AVMA, 145:787-789.

Palmer, J. S. 1964. Toxicity of Carbamate, Triazine, Dichloropropionanilide, and Diallylacetamide Compounds to Sheep. Jour. AVMA, 145:917-920.

Palmer, J. S. 1964. Oximes for Treatment of Coumaphos Poisoning in Cattle. Jour. AVMA, 145:1206-1210.

Palmer, J. S. 1965. Observations of Brahman and Hereford Cattle Sprayed with Dioxathion. Jour. AVMA, 146:221-223.

Palmer, J. S. 1965. Oxime Therapy for Cattle with Dioxathion Poisoning. Jour. AVMA, 146:697-700.

Young, J. E., Younger, R. L., Radeleff, R. D., Hunt, L. M., and McLaran, J.K. 1965. Some Observations on Certain Serum Enzymes of Sheep. Amer. J. Vet. Res., 26:641-644.

Younger, R. L., and Radeleff, R. D. 1964. Use of Pyridine-2-Aldoxime Methochloride in the Treatment of Organic Phosphorus Compound Poisoning in Livestock. Amer. J. Vet. Res., 26:981-987.

Younger, R. L. 1965. Toxicity Studies of Certain Organic Phosphorus Compounds in Horses. Amer. J. Vet. Res., 26:766-779.

Mycotic Diseases

Keeler, R. E., and Pier, A. C. 1965. Extracellular Antigens of Nocardia asteroides. II. Fractionation and Chemical Characterization. Amer. Rev. Resp. Dis., 91:400-409.

Pier, A. C. 1964. Dermatophytosis in Animals Transmissible to Man. Occupational Diseases Acquired from Animals. Univ. Mich. School Pub. Health Cont. Educ. Series 124:79-87.

Pier, A. C., and Keeler, R. F. 1965. Extracellular Antigens of Nocardia asteroides. I. Production and Immunologic Characterization. Amer. Rev. Resp. Dis., 91:391-399.

Biological Changes Associated with Neuropathological Conditions

Buck, W. B. 1965. Functional and Biochemical Changes in Brain Tissues and in Serially Sampled Cerebrospinal Fluid During Induced Convulsions in Sheep. Ann. New York Acad. Sci., 3(2):751-782.

Buck, W. B., Bond, Kathleen, and Lyon, N. C. 1965. A Technique for Permanent Cannulation of the Carotid Artery of Sheep and Goats. Cornell Vet., 55:154-163.

Proteins and Other Complex Molecules

Elmore, Jane. 1964. A Chemical Comparison of the Flagella from Two Strains of Vibrio fetus. M.S. Thesis, Iowa State University.

Microbial Antigens

Pirtle, E. C., Rebers, P. A., and Weigel, W.W. 1965. Nitrogen-Containing and Carbohydrate-Containing Antigen from Actinomyces bovis. J. Bact., 89:880-888.

Rebers, P. A., Heddleston, K. L., and Ganfield, D. J. 1965. Isolation and Characterization of a Toxic, Particulate and Protective Antigenic Fraction from Pasteurella multocida. Fed. Proc., 24(2):698.

Ruminant Digestive Tract Microbiology

Allison, M. J., Bucklin, J. A., and Dougherty, R. W. 1964. Ruminal Changes after Overfeeding with Wheat and the Effect of Intraruminal Inoculation on Adaptation to a Ration Containing Wheat. J. Animal Sci., 23:1164-1171.

Allison, M. J., Dougherty, R. W., and Bucklin, J. A. 1964. Intraruminal Inoculation and Adaptation to Grain. J. Animal Sci., 23:1205.

Allison, M. J. 1965. Phenylalanine Biosynthesis from Phenylacetic Acid by Anaerobic Bacteria from the Rumen. Biochem. Biophys. Research Communications, 18:30-35.

Allison, M. J. 1965. The Nutrition of Rumen Bacteria. Second International Symposium on the Physiology of Digestion in the Ruminant, Ames, Iowa, August, 1964. (To be published in book form, "Physiology of Digestion in the Ruminant." Edited by R. W. Dougherty et al.)

AREA NO. 8 - FOOT-AND-MOUTH AND OTHER EXOTIC INFECTIOUS DISEASES OF CATTLE

Problem. The Congress in 1948 authorized establishment of a laboratory in the United States for research on foot-and-mouth and other exotic animal diseases. The law required that the laboratory and related facilities for research and study be located on a coastal island separated from the mainland by deep, navigable waters. Plum Island was selected as the site for the laboratory on July 28, 1952. The Plum Island Animal Disease Laboratory as a U. S. Department of Agriculture venture came into existence on July 1, 1954, and since that time this laboratory has been responsible for protecting the nation's livestock industry against animal diseases of foreign origin. Foot-and-mouth disease has visited the United States on 9 occasions and each time has been eradicated. The last outbreak of foot-and-mouth disease was in 1929. Contagious bovine pleuropneumonia was eradicated in the 1880's and has not recurred since. Success in keeping these exotic animal diseases out of the United States has been due to a number of factors and a continuing vigilance by U. S. Department of Agriculture personnel.

The establishment of the Plum Island Animal Disease Laboratory and its continuing research program on exotic animal diseases has provided a laboratory in the United States where research on animal disease foreign to our soils is carried out. As new information is developed at the laboratory, it is made available to those agencies in the Department responsible for keeping out livestock animal diseases which do not occur in this country. Foot-and-mouth disease is capable of reducing our overall productivity by 25% in areas where it might become established. The disease exists in all large land areas of the world with the exception of Central and North America, Australia, and New Zealand.

Rinderpest, a disease of cattle, continues to be a serious disease problem in Africa and Asia. This disease is capable of killing 90% or more of the cattle exposed to it. Other diseases for which the laboratory is responsible include contagious bovine pleuropneumonia, Rift Valley fever, East Coast fever, and lumpy skin disease. All of these diseases continue to cause severe losses in other parts of the world. The possibilities of entry of these diseases in the United States continues, primarily because of the progressively increasing scope, speed, and extent of modern international transportation. Information developed at the Plum Island Animal Disease Laboratory is applied to the protection of the nation's livestock against foreign animal diseases.

The research continues to develop and maintain a competence for diagnosis of exotic animal diseases. Fundamental research is being conducted on biological, chemical, and physical properties of the infective agents that may be useful in prevention, control, and eradication of these diseases.

USDA AND COOPERATIVE PROGRAM

The Department at its Plum Island Animal Disease Laboratory has a continuing long-term program involving veterinarians, biochemists, biophysicists, microbiologists, and pathologists engaged in basic and applied research in this problem area. All of this research is conducted at the Plum Island Animal Disease Laboratory, Greenport, New York, except for supplemental field studies on foot-and-mouth disease vaccines which is conducted cooperatively in The Netherlands. The Department is also engaged in research under terms of an Interagency Agreement with the Assistance In Development Program, U. S. State Department, in Kenya, on contagious bovine pleuropneumonia.

The Federal scientific effort devoted to research in this area conducted solely at the Plum Island Animal Disease Laboratory, totals 23.5 professional man-years. This effort is divided among sub-headings as follows:

Studies on foot-and-mouth disease virus 2.0

Determine mechanism of antibody formation 1.0

Immune response of cattle to types and sub-types of foot-and-mouth disease virus 1.0

Quantity production of foot-and-mouth disease virus 2.0

Establishment and characterization of cell lines and cell strains 1.0

Mechanism of the interaction between foot-and-mouth disease virus molecules and host cells 2.0

Genetic biochemistry of foot-and-mouth disease virus 1.0

Effects of chemical and physical environment on foot-and-mouth disease virus 1.0

Bulk freeze drying of foot-and-mouth disease virus vaccine and antiserum 1.0

Identification, purification and chemical and physical characterization of foot-and-mouth disease virus and other exotic animal viruses 2.0

Immuno-chemical investigations of foot-and-mouth disease virus 1.5

Attenuation of representative types of foot-and-mouth disease virus 1.0

Survival and inactivation of foot-and-mouth disease virus in meat and meat by-products 1.0

Biological mechanism of natural resistance and susceptibility to foot-and mouth disease virus 1.0

Biological alteration of foot-and-mouth disease virus from continual residence in cell cultures 1.0

Morphological aspects of virus-cell relationships 1.0

Diagnostic and immunizing procedures for contagious bovine pleuropneumonia 3.0

Work was continued under a PL 480 grant to the Instituto Biologica, Sao Paulo, Brazil, for a 5-year study of tissue culture of indigenous strains of foot-and-mouth disease virus, and experimental field vaccination.

Under a PL 480 grant to the Ministry of Agriculture, Laboratories of Foot-and-Mouth Disease and Tissue Culture, Etlik, Turkey, research is under way on "Studies of Various Indigenous Types of Foot-and-Mouth Disease Virus, and the Production of a Vaccine for the Control of Foot-and-Mouth Disease in Turkey."

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Immunological Studies on Foot-and-Mouth Disease Vaccine

Chemically treated baby hamster kidney (BHK) cell culture virus used with an oil adjuvant protected steers from infection with homologous virus exposure for a six month period. The response to this inoculum in cattle, appeared to correlate the response in swine.

Foot-and-mouth disease virus degraded to small particle by acid at pH 4.8 was found to react with foot-and-mouth disease (FMD) antibody and block it to the extent it was no longer able to neutralize infectious virus. This blocking could occur with heterologous small particle (type O) as well as the homologous type (type A). These data indicate strongly that the small particle of foot-and-mouth disease virus (FMDV) may have antigens common to all of the virus types and that specificity is associated with the intact virus particle. (ADP a8-8(R)

B. Immunological Study - Mechanism of Antibody Formation

The interaction between virus and antibody was found to be time and temperature dependent. These variables may reflect differences in neutralization values as determined by suckling mice and bovine kidney cell culture assays. Higher PD_{50} values were obtained at room temperature and at 37°C than at 0-5°C over a 6-hour period of incubation. Un-neutralized, infectious virus was detected in bovine kidney cell cultures from serum-virus mixtures that were innocuous for suckling mice. This observation may also account for the discrepancy between suckling mice and tissue culture assays.

Glycidaldehyde (GDA) was determined to possess desirable stability characteristics as compared to more popular inactivants. Little loss in virucidal potency was noted over a seven month period and after storage at ambient temperatures. A substantial amount of the virus inactivating capacity of GDA remains intact, although reduced, even after 24 hours at 37°C, while after 4 hours at 37°C, the slope of the inactivation curve was similar to that produced by freshly added GDA. A more rapid rate of inactivation by .02% GDA was noted when medium containing less GDA combining constituents was used. The overall effect of extraneous materials in the medium is to lower the concentration of GDA available for virucidal action and to decrease the rate of inactivation.

Suspensions of FMDv, A-119, propagated in baby hamster kidney cells, were treated with .02% GDA for 4 hours at 37°C. The rate of inactivation indicated a departure from first-order reaction kinetics. Two phases of inactivation were noted, but it is concluded that, with appropriate interpretation of the reaction kinetics, the inactivation does lend itself to that type from which a predictable margin of safety may be determined.

Infectious and killed virus preparations were antigenically potent and produced antibody levels capable of protecting animals against high challenge doses of infectious virus. (ADP a8-10(R)

C. Immune Response of Cattle to Types and Sub-Types of Foot-and-Mouth Disease Virus

Convalescent bovine sera have been assayed for neutralizing antibody, using suckling mice and tissue culture as the assay system. Values for levels of neutralizing antibody have been consistently higher when assayed in mice than when assayed in tissue culture. Differences in the rate of inactivation of virus by sera taken at different times following infection, appears to be attributable to the type of antibody present in the serum.

Knowledge of the protection conferred by foot-and-mouth disease vaccines and understanding of available methods for evaluating the degree and duration of such immunity are essential for planning of field vaccination programs. Information can only be obtained by observations and study involving large numbers of cattle over long periods of time. An ADP representative has been conducting investigations in cooperation with Dutch Ministry of Agriculture scientists in The Netherlands where annual vaccination of cattle against types O, A, and C foot-and-mouth disease is practiced. Cattle for serological and infectivity studies are available from selected Dutch dairy herds, from certain groups of animals destined for slaughter at the Amsterdam abattoir and animals used for vaccine potency tests at the Central Veterinary Institute.

Serum antibody titers against the three types of virus persisted over a four-year period in most of the 360 cattle which had been vaccinated two or more times at annual intervals. Antibody levels remained relatively

constant from 12 or 18 months following vaccination through a four-year period. Average antibody levels against type C virus were considerably lower than against types O and A.

In general, a good correlation was observed between serum antibody level and resistance to infection. In studies using type C virus, animals challenged two weeks following vaccination were more resistant than those with comparable titers exposed 9 to 48 months post-vaccination. (ADP a8-11(R)

D. Quantity Production of Foot-and-Mouth Disease Virus

Studies were continued to develop basic information on virus-cell inter-relationships applicable to better methods for detection, assay, and production of virus in cells grown on glass and in suspension.

Strains of all 7 types of foot-and-mouth disease virus (FMDV) were characterized according to frequency distribution of plaque diameters. There was a significant relationship between plaque size in primary cultures of bovine kidney cells and infectivity for cattle by intramuscular (i.m.) inoculation. Low dosage levels of large plaque strains were superior to small plaque strains for infecting cattle by the i.m. route. No relationship existed between plaque size and infectivity when the same strains were inoculated into mice, guinea pigs, cattle, or cell cultures. An inverse relationship existed between number of plaques formed by FMDV in primary bovine kidney cell cultures, and the concentration of bovine, young calf, or agamma newborn calf serum was used in the growth medium. Fewer plaques were formed when change medium contained bovine serum.

There was no evidence of reduced susceptibility of primary cultures of bovine kidney cells to infection with FMDV when serum containing antibodies against FMDV was used in the growth medium, providing the cell cultures were washed at time of medium change and the change medium was free of serum. Failure to wash the cultures resulted in reduction of number of plaques in cultures inoculated with virus homologous to the serum. (ADP-a8-12(R)

E. Establishment and Characterization of Cell Lines and Cell Strains

Plaque formation by different strains of FMDV in a line of baby hamster kidney cells is being studied.

Studies on susceptibility of a line of pig kidney cells to infection with various strains of FMDV have been initiated. (ADP a8-14(R)

F. Mechanism of the Interaction between Foot-and-Mouth Disease Virus Molecules and Host Cells

Chemically-defined medium for baby hamster kidney cells (BHK). Studies were made on the development of a protein-free, chemically-characterized medium for the growth of baby hamster kidney culture cells on glass for use in the study of the biosynthesis of FMDV. In high multiplicity infection experiments in BHK cells of 5-hour duration, no definite medium was as satisfactory as the original growth medium containing serum and lactalbumin hydrolyzate. TB-H, glu, fortified with 0.03% glutamine, allowed the production of 0.5 log units less virus infectivity than complete growth medium, and was the best defined medium to be developed thus far. In media studied, the virus produced within 5 hours was still largely intra-cellular. Glutamine was not replaceable by lactalbumin hydrolyzate, nor by substances known to be produced from it *in vivo*, i.e., glutamic acid, proline, aspartic acid, asparagine, and the RNA bases uracil, cytosine, adenine, and guanidine. Glutamine stimulated virus production independently of the action of glucose, each chemical yielding about 1.2 log units less virus than complete growth medium. Glutamine, unlike glucose, stimulated oxygen uptake in uninfected cells by an average of 23%. In infected cells, which showed decrease in oxygen uptake, glutamine was able to enhance respiration. This apparent maintenance of cellular viability by glutamine may help explain its role in increasing viral production.

Studies were continued on the effect of chemical agents on FMDV production in cell cultures. Amantadine was tested against FMDV in baby hamster and calf kidney cell cultures. Concentrations of 25 and 250 µg/ml of agent in growth medium containing lactalbumin hydrolyzate and serum were ineffective in reducing virus yields in experiments at a high multiplicity of infection during 5 hours, and in low multiplicity experiments during 18 hours. Amantadine was ineffective in the absence of lactalbumin hydrolyzate and serum in the growth medium in high multiplicity of infection experiments, but did inhibit virus production when infection was at a low multiplicity. This inhibition could be attributed to loosening of cells from the glass surface by the toxicity of the chemical. (ADP a8-17(R)

G. Studies on Genetic Biochemistry of Foot-and-Mouth Disease Virus

Control of DNA function in cells infected with FMDV. Infection of animal cells with viruses has been shown to shut off normal cellular transcription. It was decided to examine whether this regulation could occur by a histone pathway. The histones of baby hamster kidney cell cultures were examined before and after infection with FMDV, A-119, for changes in heterogeneity, methylation, acetylation and amino acid incorporation. It was found by electrophoretic analysis that infection by FMDV rapidly increased the heterogeneity of cells with arginine rich histones. This suggested an increased histone control mechanism in infected cells. Similar changes were observed by extensive passage of noninfected cells. In support of a histone pathway for decreasing DNA transcription in infected cells, it was

observed that ^{14}C lysine and ^{14}C arginine were incorporated at a 50% greater rate into both lysine and arginine rich histones immediately after infection. Likewise, increased regulation by histone after infection was revealed by 3.5-fold decreases in the rates of acetylation (by ^{14}C sodium acetate) of the N-terminal group of the arginine rich histone and methylation (by ^{14}C methyl methionine) of the e-amino group of the lysine-rich histone.

Reaction of FMDV with Cations and Formaldehyde. Structural changes during the reaction of pure FMDV with cations and formaldehyde were deduced from ultraviolet absorbance measurements. Absorbance-time and absorbance-temperature profiles of FMDV were determined sequentially in the presence of sodium and magnesium ions and in CH_2O. It could be inferred from the profiles that FMDV broke down spontaneously at 10°C in 0.001 M Na^+ to protein and hypochromic ribonucleic acid. The latter then denatured reversibly when heated. Mg^{++} at 10^{-3} markedly suppressed the spontaneous degradation of FMDV; lower concentrations of Mg^{++} were progressively less effective. CH_2O at 0.25% appeared to cause the degradation of FMDV at 10°C at a sodium ion concentration, 0.02 M, where the virus was otherwise stable up to 53°C. CH_2O did not lower the degradation temperature as much at higher concentrations of sodium ions where the virus was known to be more stable. Plausible mechanisms were suggested for the action of heat, cations and CH_2O on FMDV. (ADP a8-18(R)

H. Effects of Certain Chemical and Physical Environments on Foot-and-Mouth Disease Virus

A 1 M concentration of hydroxylamine reduced the titer of FMDV O-M11 6-7 logs in 15-30 minutes at temperatures of 37, 23, and 4°C. The titer was reduced to the same extent by a 0.1 M concentration of the chemical at 4°C, but 28 hours incubation was required. Better immunological preparations, as tested in adult chickens and mice, were obtained when 0.25 M concentrations were used at 23 or 4°C for 6 to 18 hours incubation.

Argentine strain A-1 FMDV was inactivated with 15 minutes by 6 μg./ml. concentrations of methylene blue, neutral red and toluidine blue followed by exposure to light rays of incandescent bulbs at a temperature of 15°C. With crystal violet used similarly, there was only a loss of about 2 logs of virus titer. The methylene blue treated virus was more immunogenic than the others.

A comparison was made between cattle strains of FMDV and their counterparts after adaptation to cell cultures regarding the stabilizing action of divalent cation, Mg^{++}, at 2 M concentration and temperature of 50°C. The laboratory cattle strain, C-149, was very stable under this treatment and Argentine field strains of types A, O, and C were not as well stabilized. The cell culture adapted viruses from these four strains were entirely unstable with this treatment. This technique may serve as a means of inactivation of cell culture adapted viruses and a means for differentiating such viruses from field or laboratory strains.

At a concentration of 0.05%, acetylethyleneimine (AEI) inactivated FMDV and allowed retention of immunogenic properties. Similar treatment with beta propiolactone left a residuum of active virus. However, when the latter treatment was preceded by ultraviolet irradiation, inactivation of virus resulted and immunogenic properties were retained comparable to AEI treatment.

Foot-and-mouth disease virus, placed in an antiserum of another type as a simulated contaminant, was inactivated by the addition of 0.3% beta propiolactone. After this treatment, the antiserum retained its neutralizing antibody activity, but there was a three tube loss in complement fixing antibody activity.

The effect of 50, 5 and 0.5 µg. of glutaraldehyde on bovine kidney cell cultures was determined. Some toxic effects, as indicated by rounding of cells, were noted due to 50 µg. of the compound but no toxic effects were produced by 5 or 0.5 µg. of glutaraldehyde. No adverse effects were noted on virus multiplication at these three levels. Glutaraldehyde inactivated approximately 3 log units of FMDV at .05% but only 1 to 0.5 logs of virus at .005% and .0005% after 4 hours at 37°C, respectively. Further studies are necessary to fully evaluate this compound. (ADP a8-19(R)

I. Bulk Freeze-Drying of Foot-and-Mouth Disease Virus Vaccines and Antiserums

A study was made of freeze-drying conditions, using cell culture adapted strain A-119 FMDV with various supporting additives as the test agent. The drying conditions that gave the better results were: A residual air pressure of $\leq$100 microns, temperature of product at 22 C and condenser at -50 C, a drying time of 36 hours and a product volume of 4.0 ml. Under these conditions, dried virus stored at 4 C in flame-sealed ampules retained full infectivity for one year. The cell culture maintenance fluid was as effective in preserving virus as the additives: skim milk, sucrose, sodium glutamate or normal cattle serum. (ADP a8-20(R)

J. Identification, Purification and Chemical and Physical Characterization of Foot-and-Mouth Disease Virus and Other Exotic Animal Viruses

A. Electron Microscopy. African swine fever virus (ASFV) was grown in a stable swine-kidney cell line, and electron micrographs of thin sections of infected cells were made during various stages of viral development. Two hours after infection virus was seen within the cytoplasma. These particles appeared to be those which had been taken up from the inoculum, since no evidence was seen of virus reproduction. In subsequent thin sections taken 24, 48, 72, and 96 hours after inoculation, areas of virus formation appeared and increased in size until the cytoplasm disrupted. A few virus particles were seen in the intercellular spaces after 24 hours, while later micrographs showed many particles budding out through the cell wall. By 96 hours, a large portion of the cytoplasm had been converted to

virus, and the cell disintegrated. The process of virus release was a continuous rather than burst process. During release the particle acquired an outer membrane of cellular material. The structure of fully developed ASFV was unique in processing a very osmophilic hexagonal wall which surrounded an electron lucent region and a central nucleoid. Measurements across the virus and nucleoid ranged from 175-215 m_u and 72-89 m_u, respectively. The period during which ASFV particles were forming in the cell was consistent with the rise of infectivity and hemadsorptinin in cell culture fluids.

FMDV structure. The rotational technique of Markham et al was used to compare electron micrographs of highly purified FMDV with rotational photographs of two models most likely to represent the structure of FMDV. The purpose was to determine more accurately the capsomeric structure of FMDV. One model was a 32 subunit rhombic tricontahedron, while the other was a 42 subunit icosahedron. A comparison of the rotational images showed that the 32 and 42 structural unit models were not readily distinguished in most instances. What might be called secondary effects of the reinforcements did indicate, however, that FMDV images were more consistent with the 32-unit structure than they were with the 42-unit structure.

Gamma Irradiation of FMDV. Work has been completed on the gamma irradiation of foot-and-mouth disease virus, type A119, from baby hamster and calf kidney tissue cultures. Comparisons were made on the effect of the cobalt-60 exposure on both crude and pure virus. Virus was maintained at 0°C except during exposure in the ^{60}Co source where the temperature remained cold to the touch even after a 60 minute exposure. The change in the infectivity of both preparations of virus was monitored as well as the physical state of the pure virus as determined by ultraviolet absorbance-temperature profiles. The approximate intensity of the ^{60}Co source was 3400 rads/min. at the sample location. Virus infectivity showed appreciable resistance to gamma rays only when protective substances were present. Inactivation rates of crude and pure viruses were approximately 0.4 and 7.0 log units/hr., respectively. Addition of gelatin (0.1%) or cysteine (0.1%) did not stabilize crude virus any further, but caused pure virus to be nearly as stable to gamma rays as crude virus. From knowledge about ionizing effects of gamma rays in aqueous systems, it appears that the additives functioned by neutralizing the newly-formed free radicals and peroxides.

Hydroxymercuribenzoate (HMB) had no effect on either crude or pure FMDV. In contrast to the apparent enhancing effect of cysteine on the infectivity of non-irradiated pure virus, HMB appeared to cause some inactivation, indicating that sulfhydryl groups in the virus may be important to infectivity. From absorbance-temperature profiles on irradiated pure virus, it could be inferred that considerable degradation of virus occurred within 1 minute of exposure. At 10 minutes, it could be deduced that scission of the sugar-phosphate backbone structure of viral RNA had commenced. At 30

minutes, backbone scission was nearly complete, and destruction of purine and pyrimidine rings had commenced. At 60 minutes, both kinds of breakage appeared to be complete.

Amino Acid Composition of FMDV. Analysis has been made of the amino acid composition of FMDV, types A_{119}, O_9, and C_3 produced in baby hamster kidney cultures and purified by procedures developed previously. Analysis was also made of type A_{119} virus from cattle which had been passaged only a few times in calf kidney tissue cultures. Statistical comparisons revealed no differences at the 0.05 level of significance in the amino acid content of type A_{119} virus whether grown in baby hamster or calf cells. However, type O9 virus differed from type A_{119} in its content of alanine, leucine, tyrosine and possibly histidine. Type C_3 differed in its content of threonine, serine, alanine, valine, isoleucine, tyrosine, phenylalanine, lysine and possibly tryptophan. Type O_9 and C_3 differed from one another in their content of all the amino acids listed previously, as well as in glycine and arginine and possibly 1/2 cystine.

Application of Digital Computers to Ultracentrifugation. An investigation of the applicability of digital computers to analytical ultracentrifugation has been completed. Since Plum Island does not possess a computer, the facilities of other institutes both in the United States and England, were employed. Three major problems were examined which apply to virus research: a) determination of sedimentation coefficients from data of radial position of the solute as a function of time; b) determination of the molecular weight from solute redistribution data, and c) determination of interaction constants from summary data of sedimentation coefficients as a function of concentration. It was concluded that a) ultracentrifuge calculations which are too tedious to compute manually can be computed automatically; b) digital computers permit assessment of internal errors and the building in of safeguards; c) both linear and non-linear least squares statistics can be used, and d) certain specified criteria should be applied when using computer programs developed by others. (ADP a8-25)

K. Immuno-Chemical Investigations of Foot-and-Mouth Disease

Cattle infected with foot-and-mouth disease virus produce four, and possibly five different molecular species of antibodies. The antibody detected first is a 19S γ_1 -globulin that reaches a high level by the 7th day but is not readily detectable after about 30 days. By the 14th day, antibodies are present that have lower sedimentation rates. These have been fractionated into three or four different electrophoretic classes of antibodies. One of these antibodies is the 7S γ_2 type and it did not fix complement as well as the faster migrating antibodies. The persistence of the different antibody types is apparently dependent upon the method of exposure to virus antigen.

Conditions for testing bovine and swine serum by the complement-fixation technique were established. (ADP a8-26)

L. Survival and Inactivation of Foot-and-Mouth Disease Virus in Meat and Meat By-Products

A study of the survival of FMDV in cattle hides was prompted by the great numbers of hides that are annually imported from FMD countries. Experimentally infected cattle were slaughtered at various times after inoculation and hide samples were taken from shoulder, lumbar, inner thigh and perineal regions. The samples were shaved and aseptic precautions were taken. With all seven types of FMD, virus was regularly detected in fresh hide samples taken from 32 cattle during the period of viremia. From 6 to 28 days after inoculation, virus was found in hides of 14 of 22 cattle, with the longest persistence of 18 days. The highest virus titer found in hides was $10^{5.5}$ cell culture plaque-forming units per gram (PFU). There was no significant difference in titers of virus from the various skin areas. A study of FMDV survival in dried, salted and chemically treated hides is in progress.

The importation of various glands and tissues from FMD countries for production of biological products prompted a study of the survival of virus in the central nervous system structures. Foot-and-mouth disease virus was detected in high titers in the pituitary gland of experimentally infected cattle from the early clinical to the early convalescent stages of the disease. Virus persisted for as long as two days after viremia ended. The highest titer obtained was $10^{6.8}$ cell culture PFU/gm. and the virus titers in the pituitary were equal to or higher than those found in the blood.

Virus was also isolated from the spinal cord, pineal body, cerebrum and cerebrospinal fluid, but less frequently and, with lower titers than from the pituitary. Virus was not isolated from cerebellum, medulla or hippocampus. Additional studies are in progress on survival of virus in other endocrine glands. (ADP a8-28)

M. Studies on the Biological Mechanisms of Natural Resistance and Susceptibility of Foot-and-Mouth Disease Virus

Mice from litters consisting of 5 and 10 animals and therefore of different weights although of the same age, were equally susceptible to FMDV at 7, 14, and 21 days of age. Their response at 21 days of age indicated that some litters of mice were more susceptible than others. Extension of this work to 28- and 35-day-old mice revealed that the mice of certain litters were still susceptible while those from other litters were resistant to the virus inoculum used.

Efforts were made to learn if a relation existed between *in vitro* virus production by mouse kidney cells and the *in vivo* response of the cell donor. Single kidneys were surgically removed from adult mice, and virus production was determined in suspensions prepared from the kidneys. The recovered mice were inoculated with virus and their response was observed. A meaningful correlation between the amount of virus produced *in vitro* and the animal's response was not obtained, due at least in part to the fact that

the mice were more susceptible following the operation. Similarly, comparison of in vitro virus production with the response of litter mates of the cell donors also failed to show a consistent pattern perhaps due to the variation in response of 35-day-old mice.

Inoculation of suspensions of minced kidneys from 7- and 35-day-old mice resulted in adsorption of 80-90% of the virus over a 3 to 4 hour period regardless of the virus concentration at the start. To date, the experiments indicate that 10-20% of this virus population is a variant which is not adsorbed by these cells under the experimental conditions.

In the performance of the plaque assay, incubation of cultures with confluent cell sheets at 37, 30, or 24°C before inoculation is satisfactory if a 37°C temperature is provided during the adsorption and subsequent incubation period. Rotation after 30 minutes of adsorption is unnecessary. A 2% serum concentration in the overlay medium is as satisfactory as 10% for plaque formation. (ADP a8-29)

N. Biological Alterations of Foot-and-Mouth Disease Virus from Continual Residence in Cell Cultures

Foot-and-mouth disease virus type A, strain 119 that had undergone primary modification by chronic residence in primary calf kidney cell cultures had no practical value as an immunizing agent. Subclinical infection required to produce immunity in cattle occurred regularly only when small carefully regulated doses were given. Larger doses sometimes failed to produce subclinical infection on account of an interference factor in the virus, or produced mild clinical signs of the disease.

Significant improvement was obtained by secondary modification of the virus effected by a single passage in cattle. The secondary modified viruses were obtained from the blood of cattle with subclinical infection from the primary modified virus. One secondary modified virus consistently produced subclinical infection (viremia without clinical signs of the disease) in a wide range of dosage. Cattle inoculated with this virus resisted challenge with virulent foot-and-mouth disease virus 22 days later.

Lyophilization damaged the secondary modified virus to the extent that some inoculated animals developed signs of the disease and some failed to become immune.

Doses of these modified viruses that produced subclinical infection in cattle, produced clinical infection in swine, although the disease was mild as compared with natural infection. (ADP a8-30)

O. Morphologic Aspects of Virus-Cell Relationships

Growth rates of cell "lines" developed from cell cultures surviving infection with FMDV were apparently less than those of conventional type cell lines. One such "line" was obtained from primary swine kidney cultures and one from primary culture of a canine seminoma. Their status (primary or permanent) remains to be determined.

Three types of unmodified FMDV (A-119, C3 CANEFA and O-M11) were propagated in primary canine kidney cell cultures. Low titer virus yields indicated intermediate degree of cell susceptibility to the viruses. Higher titers of canine cell passaged viruses were obtained by subsequent passage in primary lamb testis cell cultures. (ADP a8-31)

P. Diagnostic and Immunizing Procedures for Contagious Bovine Pleuropneumonia

The value of mice and cell cultures for propagation of the etiologic agent has been investigated. In both systems, Mycoplasma mycoides will reproduce and persist but neutralization of the activity has not been successful. M. mycoides appears to be extremely sensitive to ethylene oxide gas as the growth of cultures were inhibited in media previously exposed to the gas.

A variety of diagnostic and reference materials have been produced for assistance in diagnosis of contagious bovine pleuropneumonia. These materials consist of - rabbit-immune serum, diagnostic antigens, and experimental serums produced in guinea pigs.

Work has also been conducted to develop a fraction of the organism which, when inoculated into cattle immune to contagious bovine pleuropneumonia, will cause a delayed skin reaction. The development of such a method would assist, immeasurably, in arriving at a diagnosis. (ADP a8-32)

Q. Studies on Foot-and-Mouth Disease Virus (PL 480 project)

Under a PL 480 Grant, research is being conducted on foot-and-mouth disease virus (FMDV) at the Instituto Biologica, Sao Paulo, Brazil. During a 6-months period 27 samples were collected from spontaneous cases of foot-and-mouth disease. Thirteen were positive in direct complement fixation tests. Types isolated were A, O, and C. Investigations on comparative observation on bovine and swine kidney cell cultures revealed that swine primary cultures are always highly susceptible, and the bovine cultures usually presented variable response to the virus infection. Doses which induced an extensive cytopathic effect in "normal" primary bovine cultures, produced only focci of dead cells in the resistant ones. Studies on 5 long-term bovine cultures showed that they had lost most of their primitive susceptibility to FMDV, and they had a diploid number of chromosomes and a low growth rate. Long term observations on 3 swine cell lines showed they had maintained their susceptibility to the same virus. (E3-ADP-2)

R. Studies on Various Indigenous Types of Foot-and-Mouth Disease Virus, and the Production of a Vaccine for the Control of FMD in Turkey (PL 480 project)

Under a PL 480 Grant to the Ministry of Agriculture, Laboratories of Foot-and-Mouth Disease and Tissue Culture, Etlik, Turkey, research was conducted on various types of foot-and-mouth disease virus indigenous to Turkey, and on the production of a vaccine for the control of the disease in Turkey.

SAT 1 Ova. F4-Kn9 strain virus was obtained from sheep. This SAT 1 type of foot-and-mouth disease infection was widespread in Turkey. It was easy to adapt to sheep. Work on other strains of the virus was stopped for the present. Ubetinis' method was found to be satisfactory for trypsinization of kidney tissue cells. Serums from horses, healthy unvaccinated cattle and recovered cattle were found to be satisfactory for use in calf and lamb kidney cell cultures. The entry of Type A FMD virus from Iran resulted in a delay of the work on SAT 1 until new facilities could be established. The cell culturing studies are progressing satisfactorily. (A22-ADP-8)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Immunological Investigations

Martinsen, J. S. 1964. Inactivation of foot-and-mouth disease virus by Glycidaldehyde. Amer. J. Vet. Res., 25:1417-1423.

Immune Response to Various Types and Sub-Types of FMDV

Ceglowski, W. S. 1965. Antibody response to the noninfectious 7 mu component of the virus of foot-and-mouth disease. Virology, 25:328-330.

Investigations of the Genetic Biochemistry of FMDV

Bachrach, H. L. 1964. Foot-and-mouth disease virus: Structure and mechanism of degradation as deduced from absorbance-temperature relationships. J. Mol. Biol. 8:348-358.

Bachrach, H. L. 1965. Foot-and-mouth disease virus: Structural changes during reaction with cations and formaldehyde as deduced from absorbance measurements. Virology 25:532-540.

Identification, Purification, and Chemical and Physical Characterization of FMDV and Other Exotic Animal Viruses

Bachrach, H. L., Trautman, R., and Breese, S. S. Jr. 1964. Chemical and Physical Properties of Virtually Pure Foot-and-Mouth Disease Virus. Amer. J. Vet. Res., 25:333-342.

Breese, Sydney S., Jr. 1964. An Improvement for Electron Microscopic Resolution of Polyoma Virus Capsomeres. Virology 24:125-126.

Breese, S. S., Jr., Howatson, A. F., and Chany, Ch. 1964. Isolation of Virus-Like Particles Associated with Kilham Rat Virus Infection of Tissue Cultures. Virology 24:598-603.

Polatnick, J., and Bachrach, H. L. 1964. Production and Purification of Milligram Amounts of Foot-and-Mouth Disease Virus from Baby Hamster Kidney Cell Cultures. Appl. Microb., 12:368-373.

Biological Mechanisms of Natural Resistance and Susceptibility of FMDV

Campbell, C. H. 1964. Relation of Physiologic Conditions to Variations in Susceptibility of Mother Mice to Foot-and-Mouth Disease Virus. J. Immunol. 92:858-863.

Campbell, C. H. 1965. Relationship of Donor Age to *in vitro* Production of Foot-and-Mouth Disease Virus by Mouse Kidney Cells. J. Exper. Med., 121:69-83.

AREA NO. 9 - FOOT-AND-MOUTH AND OTHER EXOTIC DISEASES OF SWINE

Problem. Foreign diseases, such as foot-and-mouth disease, African swine fever, and Teschen disease, that occur elsewhere in the world, constitute calculable potential threats to the swine industry of the United States. Foot-and-mouth disease is of particular importance because the disease frequently occurs primarily in swine from which it spreads to other susceptible species, such as cattle and other ruminants. African swine fever, which until recently was confined to wild and domestic pigs in Africa, has spread to Portugal, Spain, and France. The disease is of special concern because of its resemblance to hog cholera, with which it may be confused. Moreover, mortality from the disease approaches 100 per cent, and there is no specific preventive vaccine. Teschen disease, which causes widespread inapparent infections and occasional involvement of the central nervous system, is another of the foreign diseases to be guarded against. A disease indistinguishable from Teschen disease has appeared in England in recent years. Despite all precautions, any of these diseases may occur in the United States, as likely as not through the medium of modern, rapid international transportation. The Plum Island Animal Disease Laboratory is engaged in studies of foreign diseases of swine for the purpose of developing information for increased protection of the Nation's swine industry.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving veterinarians, biochemists, microbiologists, and pathologists, engaged in basic and applied research in this problem area. Research is being conducted on the following diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 4.0 professional man-years. This effort is divided among sub-headings as follows:

Foot-and-Mouth Disease of Swine 1.0 at the Plum Island Animal Disease Laboratory, Greenport, Long Island, New York.

African Swine Fever 3.0 at the Plum Island Animal Disease Laboratory in cooperation with the East African Veterinary Research Organization, Muguga, Kenya, and in connection with a PL 480 project in Madrid, Spain.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Foot-and-Mouth Disease of Swine

At the Plum Island Animal Disease Laboratory, studies in swine with foot-and-mouth disease virus (FMDV), type C-3 CANEFA, indicate that this virus appears to have an affinity for swine and causes extensive myocardial damage.

The modified strain tested has not been satisfactory either in quantity of endurance or antibody produced. Chemically treated baby hamster kidney (BHK) cell culture virus, combined with a Freund's adjuvant (incomplete), shows promise of protecting swine against exposure to homologous virus for at least 90 days, and in some cases, 180 days.

Recovered swine exposed to a homologous virus may become infected.
(PIADL) (ADP a9-1)

B. African Swine Fever (ASF)

Researchers at the Plum Island Animal Disease Laboratory and at the East African Veterinary Research Organization laboratory at Muguga, Kenya, report as follows:

The stable line of baby hamster kidney cells (BHK 23) is extremely prolific and ASF isolates were readily adapted to them. Virus proliferation progresses at a rate approximately double that obtained in other stable cell lines or primary cultures of pig kidney (PK) cells. Cultures in Povitsky bottles have been used to produce liter quantities of fluids containing about 10^7 $TCID_{50}$/ml. This has served as an ample source of virus for purification trials, fractionation of viral antigens and inactivation studies.

The Tengani isolate, passaged 45 times in buffy coat cultures followed by 40-45 passages in BHK 23 cells, has been modified sufficiently to cause non-fatal infections in pigs. Two of these animals have subsequently survived inoculation with fully virulent Tengani virus. Their sera contained African Swine fever virus (ASFV) antibodies which are demonstrable by complement-fixation and agar-gel diffusion precipitin tests.

African swine fever virus, Tengani, was passaged rapidly, 121 times in buffy coat cultures at daily intervals. Criteria for attenuation was the response of pigs inoculated with virus at passage levels 30, 40, 51, 63, and 121. Pigs inoculated with all passage levels were either moribund or dead within 13 days after inoculation. Neither the virulence of the virus nor the character of the lesions was modified by serial passage.

To date, 39 passages have been completed in PK 13 cell cultures with the 14th passage of the Hinde isolate in primary pig kidney cell cultures. Originally, from 7-12 days were required to obtain cytopathic changes in inoculated cultures of PK 13 cells but following 6 or 7 passages, cytopathic changes took place in 3-5 days, depending upon the concentration of the inoculum. The second passage of Hinde PK 14 virus was inoculated into pigs but showed no attenuation.

Cultures prepared with the PK 13 cell line and primary pig kidney cells had been used successfully to titrate the infectivity of the passage viruses.

Sera obtained from pigs surviving both inoculation with tissue culture passage and challenge virus were tested, likewise, but were devoid of virus neutralizing antibodies.

Lambs and guinea pigs inoculated repeatedly with Hinde tissue culture virus failed to develop virus-neutralizing antibodies.

Attempts to produce plaques using overlay medium prepared with Noble's agar, ion agar and hydrolysed starch failed, although in several instances some cytopathic effect was observed underneath the overlay.

Successive stages in the development of the virus particle propagated in PK 13 and primary pig kidney cultures have been examined in thin sections by the electron microscope. The mature particle has a hexagonal outer membrane structure (diam. 175-215 m/u) surrounding an electron lucent region and a dense nucleoid (diam. 72-89 m/u). The increase in particle production in the cell cytoplasm is consistent with both the rise in infectivity of culture fluids in pig kidney cells and the rise of the haemadsorption titer in leukocyte cultures.

Attempts to purify ASFV for studies of free virus by electron microscopy have, thus far, been unsuccessful. The virus particles are apparently intimately associated with the host tissue. All methods of separation assayed have proven to be injurious to the virus.

The Tengani isolate of ASFV grown on BHK cells was fractionated to separate the virus from the soluble noninfectious antigens. Each of the fractions was inoculated into rabbits and the sera obtained from rabbits are being tested for their ability to neutralize the Tengani virus and other isolates.

Each of the fractions will be analyzed for phosphorous, nitrogen, ribose, desoxyribose nucleic acids and carbohydrates, etc., to ascertain the biochemical identity of the fractions.

Kidneys from 2 pigs which died from an acute infection with ASFV were fractionated. When the tissue homogenate was dialyzed at pH 5.0 against a buffer of low ionic strength, a precipitate was formed which contained complement-fixing antigen. Another complement-fixing antigen was also found in the supernatant fluid. This soluble antigen can be concentrated by the addition of 50% ethanol to yield an antigen that has a complement-fixing titer in excess of 1:300 when tested against convalescent ASF swine sera. The pH 5.0 soluble fraction from ASFV-infected tissue culture fluid contained antigens which were isolate specific. Should this observation hold true for antigens derived from pig tissues, the procedure may be useful for virus typing studies.

Hemadsorption capability of ASFV could be destroyed in 60 minutes at 37 C using 0.05% B-propiolactone, acetylethyleneimine and glycedaldehyde. There was no detectable change in the complement-fixing activity following treatment.

Pepsin, trypsin, and papain at concentrations of 0.1 mg/ml., and for 3 hours at 37 C, did not alter the infectivity of ASFV. Complement-fixing activity of the virus was reduced only in the case of papain. Further enzymic studies are planned employing other enzymes and a lower pH range for pepsin.

The virus of ASF was not infective for pigs after exposure to ethylene oxide gas but pigs were infected when given challenge inoculation with 10^2 pig lethal doses of virulent ASFV.

The effect of stress, induced by FMDV, attenuated hog cholera virus (AHCV) and rinderpest virus RV) on pigs previously inoculated with attenuated African swine fever virus (AASFV) was studied. Prior to infection with these viruses, all pigs used in the trials were inoculated intramuscularly with 1 ml of ASFV Lisbon 60 passaged 81 times in pig bone-marrow cell culture. Five pigs in one group were inoculated with RV; a second and third group of 4 pigs each were inoculated with AHCV and FMDV, respectively; a fourth group of 8 vaccinated pigs was retained as controls. All the pigs were given virulent ASFV at the first signs of leukopenia and thermal reaction following AHCV, FMDV, and RV inoculations.

None of the pigs with leukopenia after challenge survived. Those pigs without leukopenia survived despite development of a febrile reaction. Seven of 13 vaccinated pigs failed to survive challenge inoculation after infection with stressor viruses. Six of the 7 pigs died with the acute form of the disease. Among controls vaccinated but not stressed, only 2 of 8 pigs died after challenge inoculation. None of the controls died with the acute form of the disease. In previous trials, using the same virus for immunization without added stressors, only 6 of 46 pigs died after challenge inoculation. These findings, although not conclusive, suggest that stressor viruses may have predisposed pigs to subsequent challenge inoculation with ASFV.

The presence of pathological alterations in lungs of 5 of 7 pigs, despite an acute course in all but one pig, further hints at the lung lesion phenomena associated with field vaccination as reported by Ribeiro and Botija.

The growth and some stability characteristics of African Swine Fever virus has been studied to aid in classifying ASFV and in standardizing laboratory tests. A survey of wild pigs near the Kitale ASF outbreak revealed that ASFV was present in wart hogs living on the farm. Mode of actual transmission could not be ascertained but the possibility of infection was there. Further studies on wart hogs from Kenya and Tanganyika have shown that many, if not all of these animals, have been previously infected and harbor ASFV in their tissues, especially the lymph nodes. Virus has not been demonstrated in the blood of any of those sacrificed as yet and in only a few has it been found in the spleen. Virulent virus inoculated into these animals apparently did not multiply.

The agar diffusion precipitation test has proved to be a very efficient practical tool for the detection of ASF antigen in tissues from pigs dying acutely of ASFV. A fluorescent antibody technique has been developed and some of its possibilities demonstrated.

An isolation of ASF virus was made from a giant forest hog and transmission of ASF between domestic pigs was shown possible by the tick Ornithodorous moubata. (PIADL and Kenya) (ADP a9-2)

Under the terms of a PL 480 agreement, research is being conducted at the Servicio de Patologia, Patronata de Biologia Animal, Embajadores, Madrid, Spain, on rapid and accurate diagnostic methods for African Swine fever. USDA scientists, working on ASF in Africa, developed a laboratory test for the diagnosis of ASF. This is based on the adsorption of red cells onto cultures of buffy coat cells. Only those cells which are infected with ASF virus will adsorb red blood cells. The occurrence of ASF in Spain, and the need to conduct diagnosis on samples suspected of being ASF, provided an opportunity to study this method of diagnosis under actual conditions. The Spanish work has shown the test to be specific for ASF. They have published variously on their application of the hemadsorption test for diagnosis of ASF. For the most part, these publications have appeared in Spanish veterinary journals and in publication media of the Office of International Epizootics (OIE), Paris, France. (Spain) (E25-ADP-4)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

African Swine Fever

Coggins, L., and Heuschele, W. P. 1965. Use of Agar Diffusion Precipitation Test in the Diagnosis of African Swine Fever. Presented FAO/OIE Meet. on African Swine Fever and Hog Cholera, Rome, May-June.

Hess, W. R., Cox, B. F., Heuschele, W. P., and Stone, S. S. 1965. Propagation and Modification of African Swine Fever Virus in Cell Cultures. Amer. J. Vet. Res., 26(110):141-146.

Heuschele, W. P., Stone, S. S., and Coggins, L. 1965. Observations on the Epizootiology of African Swine Fever. Bull. Epiz. Dis. Afr., 13.

Heuschele, W. P., Coggins, L., and Stone, S. S. 1965. Fluorescent Antibody Studies on African Swine Fever Virus. Presented FAO/OIE Meet. on African Swine Fever and Hog Cholera, Rome, May-June.

Heuschele, W. P., and Coggins, L. 1965. Isolation of African Swine Fever Virus from a Giant Forest Hog. Bull. Epiz. Dis. Afr., 13:

Stone, S. S., and Hess, W. R. 1965. The Effect of Some Chemical Inactivants on the Immunogenicity of African Swine Fever Virus. Presented at FAO/OIE Meet. on African Swine Fever and Hog Cholera, Rome, May-June.

Stone, S. S., and Heuschele, W. P. 1965. The Role of the Hippopotamus in the Epizootiology of African Swine Fever. Bull. Epiz. Dis. Afr., 13:23-28.

AREA NO. 10 - FOOT-AND-MOUTH AND OTHER EXOTIC DISEASES OF SHEEP

Problem. For the early detection of any outbreak of foot-and-mouth disease, comprehensive information regarding its effect on all susceptible species is necessary. The effect of foot-and-mouth disease (FMD) on cattle and swine has been, and is being investigated; however, little information is available pertaining to the disease in sheep. Sheep infected with FMD could serve as a source of infection and initiate the spread of the disease. Although primary research emphasis on exotic diseases of sheep at the Plum Island Animal Disease Laboratory is on FMD because of its great economic importance, other exotic diseases of sheep, such as rinderpest, sheep pox, louping ill, Nairobi sheep disease, and Rift Valley fever, are of concern to the Plum Island Laboratory because techniques and materials may be needed for diagnosis, control, and eradication on short notice and unexpectedly. Such diseases, if introduced into this country, could result in high death tolls or cause serious economic losses among susceptible sheep and other livestock. The problem is one of development of basic information applicable to protection of the nation's sheep from foreign animal diseases; development and maintenance of competence in diagnosis of these diseases, and fundamental research on the biological, chemical, and physical properties of the infectious agents that may be useful in prevention, control, and eradication of these diseases.

USDA AND COOPERATIVE PROGRAM

The Department has recently activated a continuing and long-term program involving veterinarians, biochemists, microbiologists, and pathologists, engaged in basic and applied research in some of the problems in this area.

The Federal scientific effort devoted to research in this area totals 1.0 professional man-years. This effort is divided among sub-headings as follows:

Foot-and-Mouth Disease of Sheep 1.0 at the Plum Island Animal Disease Laboratory, Greenport, Long Island, New York.

Sheep Pox. Public Law 480 funds have been made available to the Turkish Ministry of Agriculture for a 2-year study of vaccines against sheep pox prepared from tissue culture propagated virus. The Madras Veterinary College, Madras, India, has also received PL 480 funds to conduct research on an efficient vaccine for protecting sheep against sheep pox. Sheep pox is indigenous in Turkey and India.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Response of Sheep to Experimental Infection with Foot-and-Mouth Disease Virus

In studies at the Plum Island Animal Disease Laboratory, virus-neutralizing, complement-fixing, and precipitating antibodies were detected in the serums of sheep following infection with foot-and-mouth disease virus (FMDV)-A119 and persisted for more than 500 days postinoculation. The persistence of antibodies in sheep and the fact that they can be readily detected is significant from a regulatory standpoint.

Sheep infected with FMDV-C-3 CANEFA, had more severe clinical signs of disease than those infected with FMDV-A119. However, while the severity of infection with different types of virus varies, sheep appear to be less susceptible to FMDV than either cattle or swine.

Investigations have been undertaken to study the clinical and serological response of goats to infection with FMDV. These investigations have not progressed sufficiently to be reported at this time. (PIADL) (ADP all-1)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

None.

AREA NO. 11 - PARASITES AND PARASITIC DISEASES OF CATTLE

Problem. The cost of parasitic diseases to the cattle industry of the United States is estimated to be in excess of $400 million annually. Disorders caused by parasites are ubiquitous, generally insidious and often overlooked entirely. Diagnosis is difficult and successful treatments for many of these diseases are not available. Moreover, management practices to avoid spread of parasitisms and to control them are often ineffectual. The problem is to develop, through a planned, balanced program of basic and applied research, knowledge for preventing, controlling or eradicating parasitic diseases so as to provide for healthy cattle, insure adequate supplies of parasite-free beef for an expanding population, avoid or minimize economic losses caused by these diseases, and thereby contribute to a more prosperous agriculture and the national economy.

USDA AND COOPERATIVE PROGRAM

The Department has a continuous long-term program involving biochemists, microbiologists, parasitologists, pathologists and veterinarians engaged in both basic and applied studies directed to the development of measures for the solution to the high and extremely costly incidence of parasitism in cattle. Research is being conducted on parasitic diseases at the following designated locations.

The Federal scientific effort devoted to research in this area totals 19.5 professional man-years. This effort is divided among subheadings as follows:

Ecological Factors Influencing Gastro-Intestinal Nematodes of Cattle 1.0 at the Animal Disease and Parasite Research Division, Regional Animal Disease Laboratory, Auburn, Alabama, and through informal cooperation with the Georgia Experiment Station, Experiment, Georgia.

Effect of Pasture Mixtures and Pasture Management on Control of Internal Parasites 1.5 at the Regional Animal Disease Laboratory, Auburn, Alabama, and through informal cooperation with the Georgia Experiment Station, Experiment, Georgia.

Acquisition and Effects of Roundworm Parasites of Cattle as Influenced by Diet 1.0 at the Animal Disease and Parasite Research Division, Beltsville Parasitological Laboratory, Beltsville, Maryland.

Host-Parasite Relationship of Coccidial Parasites of Cattle 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Ecology and Immunology of the Cattle Lungworm, Dictyocaulus viviparus 1.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Clinical and Physiological Aspects of Roundworm Parasitism in Cattle, Including Anthelmintic Treatment 1.5 at the University of California, Davis, under a cooperative agreement with the ARS-USDA.

Investigations of Trichomonad Parasites 1.0 at the Animal Disease and Parasite Research Division Regional Animal Disease Laboratory, Logan, Utah, and under a cooperative agreement with the Utah Agricultural Experiment Station, Logan, Utah.

Host-Parasite Relationship of Intestinal Worms, Cooperia spp. in Cattle 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Epizootiological and Ecological Investigations of the Internal Parasites of Grazing Cattle 1.5 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Etiology and Immune Response of Cattle to Winter Coccidiosis 1.0 at the Regional Animal Disease Laboratory, Logan, Utah, and under a cooperative agreement with the Montana Agricultural Experiment Station, Bozeman.

Anaplasmosis of Cattle 4.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland, and through a Memorandum of Understanding and other agreements in cooperation with the State Experiment Stations in California, Illinois, Louisiana, Nevada, and State Veterinarian of Tennessee, the USDA Entomology Research Station, Kerrville, Texas, and the Delta Branch Experiment Station, Stoneville, Mississippi.

Interrelationships of Diet and Parasitic Infection in the Production of Cattle 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Histochemistry of Gastro-Intestinal Nematodes of Cattle 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Parasites of Cattle with emphasis on Stephanofilarial Species 1.0 at the Animal Disease and Parasite Research Division Regional Animal Disease Laboratory, University Park, New Mexico.

Effect of Stocking Rate and Rotational Grazing on Internal Parasitism of Cattle 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Environmental Factors Influencing Parasites and Parasitic Diseases of Economical Importance in Ruminants (Cattle, Sheep, and Alpacas) (PL-480 Peru)

Investigations on Anaplasmosis, Piroplasmosis and Babesiallosis of Cattle are under way through a PL 480 Grant at the School of Veterinary, Montevideo, Uruguay (PL 480 Uruguay)

PROGRAM OF STATE EXPERIMENT STATIONS

The State Stations have a long term program covering basic and applied aspects of the major internal parasite problems of cattle. Twelve states in the Western Region and the Department are cooperating in regional research on cattle nematode problems (W-35). Informal coordination is maintained with States in the Southern Region also working in this subject matter area.

Basic research is in progress to establish how nematodes damage the host animal, interfere with nutrition and result in disease. Research on the biochemical systems concerned with parasite metabolism and the effect of anthelmintics on these systems is providing basic information necessary in developing improved therapeutic controls. Other studies are centered on means for reducing the exposure of cattle to infective stages of parasites. Systems of grazing management and feeding procedures are being evaluated and factors which favor over-wintering survival of parasite larvae are being determined. The relationship between types of pasture forage and the degree of parasitism are being determined and the micro-climatic conditions conducive to parasite larval infectivity are being established. Immune mechanisms involved in resistance to parasites are being determined.

A number of states are engaged in studies on the nature of Anaplasma, the life cycle of this parasite, methods for immunization against anaplasmosis and procedures for eradicating the disease. Other work is in progress on the life cycle and means of controlling coccidiosis of cattle.

The total State scientific effort devoted to this research is 29.1 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Etiological Factors Influencing Gastro-Intestinal Nematodes of Cattle

The report on research conducted at Experiment, Georgia, under the auspices of the Regional Animal Disease Laboratory at Auburn, Alabama, shows the use of vermiculite as a culture medium for larvae of nematodes of ruminants was investigated. Feces containing eggs of Oesophagostomum radiatum, Cooperia pectinata, C. oncophora, Trichostrongylus colubriformis or T. axei was collected from monospecifically infected calves and cultured in either the conventional sphagnum moss or vermiculite (Terra-Lite Brand, Zonolite Company). Average larval recovery was higher from cultures made with vermiculite than those made with moss, although the differences were not significant. Larvae in sheep pellets were also cultured successfully with vermiculite. In general, vermiculite was more advantageous for routine culturing, being cleaner, more hydrophilic, more economical, and conveniently packaged. Furthermore, the granular nature of vermiculite makes it easier to mix and spread out on the water surface of a Baermann funnel.

(Alabama and Georgia) (ADP b1-6(R)

B. Host-Parasite Relationship of Coccidial Parasites of Cattle.

Scientists at the Animal Disease and Parasite Research Division's Regional Animal Disease Laboratory at Auburn, Alabama, reported the following: When 20% antiformin and 1.0 M sulfuric acid were used consecutively or separately there was very little reduction in sporulation or destruction of oocysts of Eimeria ellipsoidalis, E. auburnensis, and E. Bovis, but when the two solutions were combined, the mixture nearly completely prevented sporulation and destroyed almost all of the oocysts and contents. Half-strength solutions were not as effective, as there was no evidence of destruction and all oocysts were not prevented from sporulating. Half-and quarter-strength solutions were more effective in reduction of sporulation of oocysts of E. auburnensis than oocysts of E. bovis.

Additional data were obtained on the life cycle of Eimeria cylindrica in calves. Six young calves were inoculated with 50,000 to 240,000 oocysts of E. cylindrica to determine the prepatent period. Five of the six became infected and had oocyst outputs ranging from light to heavy. The prepatent period was 9 days postinoculation (PI) in one, and 10 days in the other four. Peak days of oocyst discharge were 12, 12, 19, 13, and 10 days PI. Patent periods lasted 8, 5, 21, 13, and 12 days. Data were obtained on the endogenous stages of the parasite in three other calves, two killed at 8 days PI and one killed at 11 days. In the first calf killed at 8 days, macrogametocytes were found in the crypts of Leiberkuhn in the lower small intestine. In the other calf killed at 8 days PI, immature macrogametocytes measuring up to 19.5 μ were found in the last portion of the small intestine in the glandular crypts.

Two calves inoculated with oocysts of Eimeria canadensis were killed at 9 and 16 days PI to obtain information on the endogenous stages of the life cycle. At 9 days PI, immature schizonts were found in the small intestine in the apices of villi one foot below the stomach and at 24 ft. above the ileocecal valve. At 16 days PI, merozoites ranging up to 18.2 μ were found in great numbers from C plus 42 ft. to C plus 6 feet. Schizonts were most numerous at C plus 12 to C plus 24. They were macroscopic and were located in the lacteals of the villi, resembling those of E. bovis and E. auburnensis. Microgametocytes resembled those of E. auburnensis; the microgametes were arranged peripherally and invaginated at places.

(Auburn, Alabama) (ADP b1-23(Rev.)

C. Clinical and Physiological Aspects of Roundworm Parasitism in Cattle including Anthelmintic Treatment.

The School of Veterinary Medicine, University of California, Davis, under a cooperative agreement with the USDA, reports on their investigations as follows:

Anthelmintic Studies (a) Disophenol (DNP), an excellent anthelmintic against a narrow spectrum of nematodes, was found to have significant action against Fasciola hepatica, the common liver fluke of sheep and cattle. (b) Haloxon at 50 mg/kg in both sheep and cattle was found to remove greater than 90% of the more important species of gastrointestinal parasites in California. (c) Field trials with Thibenzole indicated that a dosage of 3 grams/100 lbs. was equal to that of 5 grams/100 lbs. in cattle as a prophylactic treatment.

Physiological Studies (a) Water balance studies indicated a marked (50%) reduction in water intake and output of cattle suffering from gastrointestinal parasitism. It was further noted that a much greater reduction (dehydration) of the extracellular water compartment occurs than is indicated by the plasma volume.

Development of an experimental model for laboratory study of physiological alterations. Data so far collected indicate that Obellscoides cuniculi infection in the rabbit may be valuable as a model for studying many of the alterations which occur in cattle as a result of gastrointestinal parasitism. (California) (ADP 81-25)

D. Investigations on Trichomonad Parasites.

Studies conducted at the Division's Regional Animal Disease Laboratory at Logan, Utah, included the production of antisera in rabbits to six strains of Trichomonas foetus. Antisera was produced in fiscal year 1964 to two strains of T. foetus, and in 1965 to four additional strains which were acquired from widely divergent sources in this country and in Europe. The antisera were produced by two graded series of five intravenous inoculations of live, washed organisms given twice weekly with a three-week interval between the two series.

Quantity of agglutinating antibody was determined by exposing live, washed trichomonads to various dilutions of the antiserum in Ringers solution. Each trichomonad was tested against its homologous antiserum and against each of the other five antisera, and each antisera was tested against its homologous trichomonad and each of the other trichomonads. Homologous titers were 20480, 20480, 5120, 5120, 2560, and 1280 which, with the exception of the 1280 titer, indicates good strong reactions. Heterologous titers ranged from 160 to 20480.

Preliminary analysis of the agglutination results indicates there are antigenic differences in the various strains of T. foetus.

Gel diffusion studies were made comparing the six strains by the Ouchterlony gel diffusion technique. The suspended particulate material in the antigen varied in size, and some of the crude preparations plugged the gel preventing migration of antigens and subsequent formation of precipitin lines.

After centrifugation of the antigen at 37,000 gravities, an antigen of considerably better quality was produced. With the improved crude antigen, precipitin lines formed indicating the presence of four to seven antigen-antibody systems. Use of the improved antigen has shown a varying number of precipitin lines form by reaction of the medium ingredients and bovine serum which is in the medium with the antiserum.

Tests made on bulls from a locality in Utah which has been troubled by trichomoniasis for the past seven years and with which we have worked closely, revealed no trichomoniasis at the present time. (Logan, Utah) (ADP b1-26)

E. Host-Parasite Relationship of Intestinal Worms, Cooperia species, in Cattle.

Reported research from the Division's Regional Animal Disease Laboratory, Auburn, Alabama, showed that cattle may be immunized against pathogenic worm parasites by controlled inoculations with closely related species. Three species of Cooperia--C. punctata, C. pectinata, and C. oncophora--are common parasites of cattle. The latter species is relatively non-pathogenic to calves, whereas the other two species are equally harmful. Calves inoculated with C. oncophora have become almost completely immune to challenge inoculation with C. pectinata and partially immune to C. punctata. Individual animals, however, failed to become immune to subsequent challenge with the original species and these calves were also unable to resist challenge with the related species.

(Auburn, Alabama) (ADP b1-27)

F. Epizootiological and Ecological Investigations of the Internal Parasites of Grazing Cattle.

Scientists at the Beltsville Parasitological Laboratory reported that malnutrition, experimentally produced in calves from 12 to 17 weeks of age, prior to infection with gastrointestinal nematodes and resultant stunting, influenced the level of parasitism acquired by them while grazing pastures, as indicated by higher worm-egg counts than those of similarly exposed full-fed calves. The driest summer on record complicated the interpretation of subsequent postmortem data insofar as determining the effects of malnutrition on the development of nematode parasitism. However, it presented an unusual opportunity to study the epidemiology of the parasitism in question under drought conditions.

Heavy contamination of the pastures with manure containing large numbers of nematode eggs during the drought led to the development of very heavy concentrations of infective larvae on the forage during the cooler weather of early fall which followed a short period of normal rainfall. The continued grazing of these heavily contaminated areas during the mild but moderately dry conditions of late fall led to extremely heavy infection of the cattle with an average of 249,000 worms per animal. The predominant

species was Ostertagia ostertagi, the medium stomach worm. This finding indicated that its free-living stages were able to survive conditions that were lethal to those of other species.

The ability of eggs of the beef tapeworm (Taenia saginata of man) to cause formation of cysts (Cysticercus bovis) in the muscles of cattle apparently was reduced rather markedly by exposure to 50,000 r, 100,000 r and 200,000 r of x-rays and in proportion to the amount of exposure. An animal that had been vaccinated with eggs exposed to 200,000 r, and was given a large dose of normal eggs subsequently, was found to be free of cysts. An average of about 3,300 cysts developed in two unvaccinated controls that received an identical dose of normal eggs. (Beltsville, Maryland) (ADP b1-28)

G. Etiology and Immune Response of Cattle to Winter Coccidiosis.

Reports on research conducted at the Division's Regional Laboratory at Logan, Utah, show that six experiments were conducted which involved coccidial infections with Eimeria bovis or Eimeria zurnii in Holstein-Friesian calves. One experiment dealt with the effect of prolonged low-level inoculations with sporulated oocysts of Eimeria bovis on the development of immunity in calves. Ten, 100, or 15,000 oocysts/day were given calves for 60 days. Clinical signs were exhibited only in calves receiving 15,000/day. The highest oocyst discharge also occurred in this group, but the number of days oocysts were discharged was about the same in all groups. All groups of calves exhibited resistance to reinfection when challenged with 500,000 oocysts at the end of 60 days inoculation.

Three experiments involving calves inoculated with Eimeria zurnii were completed. All were designed to gain information on methods of producing reliable experimental infections. Cortisone injections were used unsuccessfully in an attempt to suppress the immunogenic mechanism in the calf and allow the coccidia to invade the host. The sporulated oocysts were treated in such a way that the exterior wall would be dissolved, thus making the oocyst more susceptible to digestive fluids, including enzymes. This technique was also unsuccessful.

In the second experiment both sporulated and unsporulated oocysts were irradiated at 10,000 r, 50,000 r, 75,000 r, or 100,000 r in a cobalt-60 source. In the first part of this experiment inoculation of calves with sporulated oocysts produced results similar to those reported last year and to those reported above. However, the results were more conclusive. A challenge inoculation after recovery from the first inoculation indicated that immunity against reinfection was present in the calves which had previously exhibited clinical signs and discharged oocysts, i.e., calves receiving oocysts irradiated at 10,000 r. Little or no resistance was observed in the other 3 groups of calves. These results indicate that irradiated oocysts have no special value as immunological agents against coccidiosis caused by Eimeria bovis. (Logan, Utah)

Studies in cooperation with the Utah State University at Logan are reported as follows:

Description of the sporulated oocysts and sporozoites of four species of bovine coccidia. The sporulated oocyst is the stage of the coccidial life cycle most favorable for identification as to species. The bovine coccidia have been described only incompletely with respect to this stage. The description, including drawings, have been completed of the sporulated oocysts and free sporozoites of the four species most common in the Logan area, namely, E. bovis, E. zurnii, E. ellipsoidalis, and E. auburnensis. The free sporozoites were obtained for observation by causing the oocysts to excyst in vitro. The sporozoites of E. zurnii were found to have only one relatively small refractile granule in each sporozoite instead of two relatively large refractile granules as in each of the other three species. This morphological difference may be associated with the peculiar epidemiological pattern exhibited by E. zurnii, for example, its association with "winter coccidiosis" and the difficulty of inducing experimental infections with this species.

Cytological study of coccidia. A cytological study of the stages of Eimeria bovis and other bovine species of coccidia was undertaken to obtain fundamental information useful in developing methods of prevention and control of coccidia. It was necessary to section oocysts because of the impermeability of their wall. Work has demonstrated its feasibility.

First-generation merozoites of Eimeria bovis were obtained in large numbers from mature schizonts, after concentration of these by repeated washing and sedimentation. The appearance of living merozoites, as well as their flexing and gliding movements, were described with the use of the phase-contrast microscope. In specimens stained with protargol the anterior portion of the body was found to have a cap-like covering with a terminal pore, and a median rod-like structure. Prominent granules occurred in the posterior 2/3 of the body, with one granule characteristically located at the posterior extremity. The nucleus was in the posterior 1/3 of the body. In Feulgen and acridine orange preparations the chromatin was arranged as a ring at the periphery of the nucleus; at irregular intervals there were coarse clumps, usually 3 to 5 in number. Numerous small glycogen granules were present in the posterior 2/3 of the body. No sudanophilic lipids were demonstrated. The entire body of each merozoite showed a diffuse positive reaction with the ninhydrin method. These merozoites were found to be similar in certain morphological features to Toxoplasma gondii.

Nitrofurazone as a prophylactic agent against experimental bovine coccidiosis.

In 3 experiments, each with 12 calves about 2 months old, the administration of nitrofurazone in the feed at 5.0 or 7.5 mg/kg of body weight daily for 6 weeks, beginning 4 days before inoculation of 50,000 to 100,000 Eimeria bovis oocysts, did not prevent the occurrence of coccidiosis. In each of these experiments the calves were allotted to 3 groups, each including 2

inoculated calves housed in the same pen with 2 uninoculated calves. Little or no infection was observed in the uninoculated calves. In 2 of the 3 experiments, sporulation of oocysts apparently did not occur in the pens during the course of the experiments because of low temperatures; in the 3rd experiment the calves evidently had some degree of immunity as a result of natural infections. The weight gains of the treated calves were not consistently different from those of the untreated calves, but the calves given 7.5 mg/kg made smaller average weight gains than those given 5.0 mg/kg. The results of 1 experiment with 8 calves, 2 months old, indicated that nitrofurazone in the feed at 10 mg/kg for 3 weeks beginning 1 week before inoculation of 100,000 oocysts each of E. bovis and Eimeria zurnii, had only questionable value in preventing coccidiosis. In 1 experiment with 6 calves about 4 months old, nitrofurazone administered in gelatin capsules at 30.0 mg/kg for 4 days starting 15 days after inoculation of 100,000 E. bovis oocysts, was effective in controlling coccidiosis.

Amprolium for control of coccidiosis in calves. Sixty-nine calves were used in 6 experiments to determine the efficacy of amprolium in controlling Eimeria bovis infections. In one of these experiments, 3 additional calves were treated with ethopabate. In each experiment three or four groups each of 3 calves about 2 weeks old, were inoculated with 50,000 or 100,000 oocysts; two or three of these groups were given liquid amprolium in the milk. In each of three experiments, one group of 3 calves was left uninoculated until 30 days after the original inoculation, then all groups were challenged with 1 million oocysts. In five experiments, treatment at 16.25 mg/lb for 21 days, beginning on the day of inoculation, provided good to excellent control of coccidiosis, as did such treatment at 65 mg/lb and 10 mg/lb in two experiments.

In four experiments, calves treated at 65 mg/lb for 5 days beginning 13 days after inoculation, had less severe signs of coccidiosis and discharged fewer oocysts than untreated calves, but the results of this treatment were not as good as those of the 21-day treatments. In one experiment, treatment with amprolium at 65 mg/lb or ethopabate at 1 gram/lb for one day, 13 days after inoculation, had little or no effect on the infections. Calves which had been treated had less severe infections after challenge than did controls not previously inoculated. (Logan, Utah) (ADP b1-29)

H. Investigations of Anaplasmosis

Research at the Beltsville Parasitological Laboratory has shown that a partially purified antigenic protein has been isolated from red blood cell hemolysates obtained from cattle with acute anaplasmosis. Concentration, characterization and immunogenic studies on the material are under way.

Thin-section studies on the ultra-structure of A. marginale have revealed that the parasite contains, in the bovine red blood cell, from one, to at least six, smaller organized units or bodies. The detailed structure of these sub-units has not been clearly demonstrated. A mild type of

A. marginale, naturally occurring in the United States, has been observed to be of similar pathogenicity to A. centrale, the so-called vaccine type used in Africa and elsewhere for premunition.

Field tests are in progress to determine if a "dead Anaplasma" antigen will protect cattle against natural exposure in a degree sufficient to prevent economic losses from the disease. (Beltsville, Md.) (ADP b1-30)

I. Histochemistry of Gastro-Intestinal Nematodes of Cattle

Research work at the Division's Regional Animal Disease Laboratory at Auburn, Alabama, was reported as follows:

Studies conducted on the histochemistry of the host response to the presence of larval nodular worms, Oesophagostomum radiatum, in the wall of the small intestine of the calf have demonstrated that the presence of these larvae is associated with a decrease in the connective tissue protein collagen and an increase, real or apparent, in glycoprotein around the site of the larval worms. These alterations in the chemistry of the tissue of the calf appear early in the infection and disappear as healing is completed.

The presence of the larval worms is also associated with an increase in the activity or amount of the enzymes alkaline phosphatase, acid phosphatase, and non-specific esterase in the vicinity of the lesion. The increase in these enzymes is apparently associated with metabolic changes in the cells of the host as they respond to the presence of the parasites. Infection by the young nodular worms produced no effect on the distribution and abundance of the enzyme leucine aminopeptidase during the stages of the infection studied.

Ketoenolic lipoids, originally discovered in certain other animals by the Japanese scientist Yukio Hamazaki, were found for the first time in the tissue of cattle and nematodes. The amount of these chemicals is increased around young nodular worms in the wall of the small intestine during some, but not all stages of nodular worm disease in cattle. These same chemicals also occur in the intestines of young nodular worms.

(Auburn, Alabama) (ADP b1-32)

J. Parasites of Cattle with Emphasis on Stephanofilarial Species

Studies made at the Division's Regional Animal Disease Laboratory at University Park, New Mexico, have shown that Stephanofilaria stilesi is a worm parasite which causes extensive sores on the skin of cattle. This chronic condition is called "stephanofilarial dermatitis." It is widespread in the United States and other parts of the world and is of much economic importance. Research based on natural and experimental infections at the University Park Field Station proved that the disease is transmitted by horn flies. Effective medicinal treatment directed at the worms in the

lesions is not yet available. Perhaps diligent control of horn flies would result in a lower incidence of the disease.
(University Park, New Mexico) (ADP bl-33)

K. Effect of Stocking Rate and Rotational Grazing on Internal Parasitism in Beef Cattle

This work was done at Experiment, Georgia, under the auspices of the Division's Regional Animal Disease Laboratory at Auburn, Alabama. The report shows that experiments suggest that rotational grazing results in increased rate of stocking of the pastures and consequently increases parasitism in beef cattle. Two lots of winter temporary pasture were stocked at the same time - one was grazed continuously and the other was grazed on a four-way rotational system. A third lot was also rotationally grazed, but the stocking rate varied with the carrying capacity of the pastures. The steers from the two rotationally grazed groups had more worms and made a lower average daily gain. The steers grazed rotationally where the stocking rate varied had the greatest number of worms. Although various factors may be responsible for an increase in parasitism, the increased stocking rate is very likely the most significant factor.
(Experiment, Georgia) (ADP bl-34)

L. Under a PL 480 grant to the School of Veterinary Medicine, University of San Marcos, Lima, Peru, research is being conducted on Environmental Factors Influencing Parasites and Parasitic Diseases of Economical Importance in Ruminants (cattle, sheep, alpacas). This project was reviewed during 1964 by a Department scientist who visited the Principal Investigator in Peru. The reviewer explained the use of several methods adaptable under conditions in Peru for improvement of the investigations.

The accomplishments for the third year of research are 1) collection of climatic data in relation with the seasonal incidence of parasitic diseases of livestock; 2) preparation of a calendar of treatment and control of parasitic diseases of livestock, according with the management and seasonal occurrences of these diseases; 3) a check list of parasites identified in our laboratory of parasitological research, during the period 1961 to 1964; 4) bionomic of fresh water snails, transmissors of Fasciola hepatica, and 5) a pamphlet on Parasites and Parasitic Diseases of Lamb pacos (Alpacas) in Peru. (PL 480) (S8-ADP-1)

Diagnosis and Methods of Prevention and Treatment of Anaplasmosis, Piroplasmosis and Babesiellosis of Cattle and Further Characterization of the Causative Agents. These investigations were conducted under a PL 480 grant to the School of Veterinary Parasitic Diseases, Montevideo, Uruguay. The work involved studies on 1) Tick development in vitro - Results - Nymph stage was achieved. Nymphs were kept alive for a period of 45-50 days in vitro. 2) Tissue cultures from bovine infected with Babesia bigemina - Results - Dermal tissue, spleen and brain were studied. 3) Tissue culture from bovine infected with Anaplasma marginale and uninfected bovine -

Results - Primary cultures showed several macrophages with parasited red cells. 4) Electrophoretic studies of bovine serum - Results - In the group inoculated with Anaplasma centrale serum protein variations occurred. The variations in general appeared in irregular form from one animal to another, differently to what occurs in anaplasmosis by A. marginale. 5) Electrophoretic studies on bovine serum - Results - Serum protein of cows infected with A. centrale and challenged with A. marginale were studied in 22 animals: a) pre-patent period - during this period only a decrease of alpha-globulin value was observed, b) patent period - at the beginning of this period, value of beta-globulin increased - remained until incidence of A. marginale, then returned to pre-inoculation value, gamma-globulin and total proteins decreased, and alpha-globulin in this period returned to pre-inoculation value. c) when red blood cells decreased under one percent; total serum protein and gamma increased, while albumin alpha and beta-globulin remained at pre-inoculation levels. d) no significant variations occurred at serum protein levels in the control cows free from A. centrale and A. marginale.
(PL 480 Uruguay) (S9-ADP-1)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Etiological factors influencing gastro-intestinal nematodes of cattle

Bizzell, William E., and Ciordia, Honorico. 1965. Dissemination of infective larvae of Trichostrongylid parasites of ruminants from feces to pasture by the fungus Pilobolus spp. Jour. Parasitol. 51:184.

Ciordia, Honorico, Bizzell, William E., and Porter, Dale A. 1965. Effect of culture temperature and age on the infectivity of larvae of Trichostrongylus axei and T. colubriformis in rabbits and guinea pigs. Jour. Parasitol., 51(2,Sec.2):48.

Porter, Dale A., Ciordia, Honorico, and Bizzell, William E. 1965. The use of vermiculite as a culture medium for larvae of nematode parasites of ruminants. Jour. Parasitol., 51(2,Sec.2):47.

Host-Parasite Relationship of Coccidial Parasites of Cattle

Davis, Leonard R., and Bowman, George W. 1964. Observations on the life cycle of Eimeria bukidnonensis Tubanqui, 1931, a coccidium of cattle. Jour. Protozool., 11(Suppl):17.

Smith, Willard N., and Davis, Leonard R. 1965. A comparison of dry and liquid feeds as vehicles for coccidial infection of cattle and sheep. Amer. J. Vet. Res., 26:273-279.

Ecology and Immunology of the Cattle Lungworm, Dictyocaulus viviparus

Lucker, J. T., Vegors, H. H., and Douvres, F. W. 1964. Immunization against the cattle lungworm: Oral vaccination with infective Dictyocaulus filaria larvae. Proc. Helm. Soc. Wash., 31:153-158.

Clinical and Physiological Aspects of Roundworm Parasitism in Cattle, including Anthelmintic Treatment

Baker, N. F. 1964. The Evaluation of Anthelmintics for Nematodes using in vivo Tests in Small Laboratory Animals. Proc. of Symposium--The Evaluation of Anthelmintics, (E.J.L. Soulsby, ed.):18-26. Merck, Sharp and Dohme International, New York.

Baker, N. F., and Douglas, J. R. 1965. Anthelmintic Activity of Haloxon in Lambs. Amer. J. Vet. Res., 112:651-653.

Douglas, J. R. 1964. The Evaluation of Anthelmintics in Cattle. Proc. of Symposium--The Evaluation of Anthelmintics, (E.J.L. Soulsby, ed.): 108-113. Merck, Sharp and Dohme International, New York.

Host-Parasite Relationship of Intestinal Worms, Cooperia Species in Cattle

Herlich, Harry. 1964. A simple method for collecting large numbers of live gastrointestinal nematodes from large animals. Amer. J. Vet. Res., 25:1772-1773.

Epizootiological-Ecological Investigations of the Internal Parasites of Grazing Cattle

Lucker, J. T., and Vegors, H. H. 1965. Vaccination against beef measles. Jour. Anim. Sci., 24:284.

Investigations of Trichomonad Parasites

Fitzgerald, Paul R. 1965. The Results of Parenteral Injections of Sporulated or Unsporulated Oocysts of Eimeria bovis in Calves. J. Protozool. 12(2):215-221.

Fitzgerald, Paul R. 1964. Effect of Bovine Coccidiosis on the Blood Serum Sodium and Potassium Levels. J. Parasitol. 50(3, sec.2):52-53.

Hammond, Datus M., and Fitzgerald, Paul R. 1964. Life Cycle of Eimeria bovis, a Coccidium of Cattle. J. Parasitol. 50(3, sec.2):47.

Jensen, E. A., and Hammond, D. M. 1964. A morphological study of trichomonads and related flagellates from the bovine digestive tract. J. Protozool. 11:386-394.

Johnson, A. Earl. 1964. Incidence and Diagnosis of Trichomoniasis in Western Beef Bulls. Jour. AVMA 145(10):1007-10.

Etiology and Immune Response of Cattle to Winter Coccidiosis

Andersen, F. L., Lowder, L. J., Hammond, D. M., and Carter, P. B. 1965. Antibody production in experimental Eimeria bovis infections in calves. Exper. Parasitol. 16:23-35.

Hammond, D. M. 1964. Coccidiosis of cattle. Thirtieth Faculty Honor Lecture, The Faculty Association, Utah State University, Logan. 35 p.

Hammond, D. M., and Ernst, J. V. 1964. Cytological observations on Eimeria bovis merozoites. J. Protozool. 11(suppl.):17-18

Hammond, D. M., Fayer, R., and Miner, M. L. 1965. Amprolium for control of experimental coccidiosis in calves. J. Parasitol. 51(suppl.):55.

Hammond, D. M., Sayin, F., and Miner, M. L. 1965. Nitrofurazone as a prophylactic against experimental bovine coccidiosis. Amer. J. Vet. Res., 26:83-89.

Nyberg, P. A., and Hammond, D. M. 1964. Excystation of Eimeria bovis and other species of bovine coccidia. J. Protozool. 11:474-480.

Anaplasmosis of Cattle

Amerault, T. E., and Roby, T. O. 1964. An exo-antigen of Anaplasma marginale in serum and erythrocytes of cattle with acute anaplasmosis. Amer. J. Vet. Res., 25(109):1642-1647.

Anthony, D. W., Madden, P. A., and Gates, D. W. 1964. Anaplasma marginale Theiler observed in the gut and excreta of Dermacentor andersoni Stiles (=D. venustus Banks). Amer. J. Vet. Res., 25(108):1464-1472.

Interrelationship of Diet and Parasitic Infection in the Production of Cattle

Dixon, Carl F. 1965. Failure of infective trichostrongyloid larvae to penetrate rabbit skin. Jour. Parasitol., 51:145-148.

Dixon, Carl F. 1965. Infection of domestic rabbits and gerbils with Trichostrongylus affinis, Graybill 1924. Jour. Parasitol., 51:299.

Herlich, Harry and Dixon, Carl F. 1965. Growth and Development of Paraspidodera uncinata, the cecal worm of the guinea pig. Jour. Parasitol., 51:300.

Histochemistry of Gastrointestinal Nematodes of Cattle

Frandsen, John C. 1964. Serial Sections; Simplified handling of paraffin ribbons on a floating-out bath. Stain Tech., 39:279-282.

Frandsen, John C. 1965. The occurrence of some carbohydrates and collagen in the nematode Obelescoides cuniculi (Graybill), a parasite of rabbits. Trans. Amer. Micr. Soc., 84:154-155.

Frandsen, John C. 1965. The nonenzymic histochemistry of early Oesophagostomiasis and Ostertagiasis in cattle. Jour. Parasitol.,51:175-9.

Frandsen, John C. 1965. Histochemical studies on the enzymology of the lesion in bovine oesophagostomiasis. Jour. Parasitol., 51(2 Sec.2):55-56.

Frandsen, John C. 1965. Observations on the Ketoenolic substances and Ketoenolic lipoids of Hamazaki in larvae of Oesophagostomum radiatum and in the tissues of the host. Jour. Parasitol., 51(2, Sec. 2):56.

The Effect of Stocking Rate and Rotational Grazing of Internal Parasitism in Beef Cattle

Ciordia, H., Bizzell, W. E., Baird, D. M., McCampbell, H. C., and White, P. E. 1964. Effect of rotational grazing systems on gastro-intestinal nematodes in beef yearlings. Amer. J. Vet. Res., 25:1473-1478.

AREA NO. 12 - PARASITES AND PARASITIC DISEASES OF SWINE

Problem. Parasitic diseases have been estimated to cost the swine industry of the United States at least $200 million annually. These diseases for the most part are cosmopolitan. Subclinical infections are the most frequent type and the most costly, yet they are generally so difficult to recognize that they often are overlooked entirely. Diagnosis is difficult, and successful treatments for many of these parasitisms are not available. Moreover, management practices to avoid the spread of parasitisms and to control them are often ineffectual. The problem is to develop, through a planned, balanced program of basic and applied research, knowledge for preventing, controlling, or eradicating parasitic diseases so as to provide for healthy swine, insure adequate supplies of parasite-free pork for an expanding population, avoid or minimize economic losses caused by these diseases, and thereby contribute to a prosperous agriculture, a sound national economy, a high standard of living, and a healthy population.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving parasitologists, veterinarians, biochemists, microbiologists, and pathologists engaged in basic and applied research in this problem area. Research is being conducted on the following diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 5.2 professional man-years. This effort is divided among sub-headings as follows:

Bionomics and Pathogenicity of the Swine Whipworm 1.0 at the Beltsville Parasitological Laboratory.

Swine Kidney Worms 0.1 under a cooperative agreement with the North Carolina Agricultural Experiment Station at Raleigh.

Investigations of Trichinella spiralis 1.0 at the Beltsville Parasitological Laboratory.

Pathogenic Role of the Intestinal Roundworm 0.1 under a cooperative agreement with the Nebraska Agricultural Experiment Station at Lincoln.

Strongyloides ransomi Infections in Baby Pigs 1.0 at the Swine Parasite Laboratory, Tifton, Georgia

Biochemical and Other Aspects of the Host-Parasite Relationship in the Development and Severity of Helminthiasis in Swine 2.0 at the Beltsville Parasitological Laboratory.

PROGRAM OF STATE EXPERIMENT STATIONS

Several States have work in progress aimed at providing improved control of swine roundworms. Efforts are centered on immunization or treatment procedures that will prevent tissue damage caused by migration of ascarid larvae through vital organs. Soil sterilants are under evaluation to find a practical method for destroying infective ascarid eggs.

Treatments are being developed to control damage due to migration of the swine threadworm through body tissues. Cooperative studies are in progress with the Department to evaluate management practices as a means for controlling swine kidney worms.

6.4 professional man-years of scientific effort are involved in swine parasite research at the States.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Swine Whipworm (Trichuris suis)

At the Beltsville Parasitological Laboratory, research disclosed that the eggs of Trichuris suis, the swine whipworm, were infective to susceptible pigs after an exposure of 8 years and 11 months, to outdoor conditions on the surface of, and buried at depths of 4 and 8 inches, in sandy loam soil in the vicinity of Beltsville, Maryland. The constant presence of moisture in the soil of the experimental plots may have been partially responsible for their survival. During the period of observation the temperature at the surface of the soil ranged from 5°F in winter to 101°F in summer.

(BPL) (ADP b2-10)

B. Swine Kidneyworm (Stephanurus dentatus)

Under a cooperative agreement research workers at the North Carolina Agricultural Experiment Station, Raleigh, report that three specific-pathogen-free (SPF) gilts were bred and isolated on pastures free of swine kidney worm contamination. Several days before anticipated farrowing, they were moved to cleaned isolation units and administered dichlorvos to remove any possible gastrointestinal parasites. Three consecutive fecal examinations proved negative. Offspring of these gilts were weaned at 5 weeks of age and remained in the isolation units. Pigs obtained from other sows and maintained on colostrum-free diets were placed in similar isolation units.

At 8 weeks of age a total of 18 pigs were infected with a single oral dose of infective larvae of Stephanurus dentatus. Dose levels were 10,000, 20,000, 30,000, 40,000, and 50,000 larvae per group. A comparable control group was maintained parasite-free.

Haematological and chemical studies conducted consisted of the following: 1) composite eosinophil count utilizing the modified Pilot technique; 2) sedimentation rate by the Winthrobe method; and 3) serum glutomic pyravic

transaminase determined as described in Sigma Chemical Co. Tech. Bulletin No. 410.

Results of these studies were: 1) Eosinophil counts began to rise approximately one month post-infection and reached peak levels approximately 6 weeks post-infection. These peak levels remained thusly for nearly 2 months and then began a gradual decline. 2) Sedimentation rate was not affected. With data obtained in human medicine, following extensive liver pathology, sedimentation rates are known to increase. This was not found to be true in light of massive liver damage inflicted by kidney worm larvae. 3) Serum transaminase values proved inconclusive. Fluctuation in values were not correlated to pathological changes or stages of infection. This test system does not seem to predict evidence of liver pathology as one would expect.

Pigs from each dose level were necropsied and colored photographs were made to record liver damage. Relationship between level of infection and liver damage is being established. Tissues were taken for histological examination. No larvae were recovered from liver or other organs. It has been our experience that no larvae are recovered from animals with less than 4 months infection. (North Carolina) (ADP b2-11)

C. Trichinosis (Trichinella spiralis)

In research studies at the Beltsville Parasitological Laboratory, trichinae, passed in the feces of pigs during the 3-day period following the administration of an initial dose of the infective larvae of Trichinella spiralis, were found to be infective to three comparable non-trichinous pigs which were fed feces of the donor pigs during this period. Transmission also occurred during the 3-day period following the administration of the second dose of larvae to the donor pigs, 30 days after the initial infection. In this instance, all nine of the non-trichinous pigs became infected, including six that were penned separately from the donor pigs and were hand-fed feed mixed with feces from the donor pigs. This finding indicated that infective trichinae were probably present in greater numbers in the feces of pigs that had become resistant to infection with the parasite following a previous infection. Of significance was the finding that 2 of the 3 non-trichinous pigs penned with the donor pigs from the fourth to the twenty-ninth day after reinfection also became trichinous. In this instance transmission probably occurred through the ingestion of adult females containing infective embryos, which would undoubtedly be passed in larger numbers by resistant pigs than by susceptible swine.

The presence of 122 pounds of large cuts of pork (hams and shoulders) in a home freezer with a capacity of 300 pounds of meat, more than doubled the time required to destroy trichinae in small packets of pork chops frozen at the same time, when the freezer was adjusted to maintain a temperature of 0°F. This phenomenon did not occur when the pork chops were frozen with approximately the same total quantity of pork present in the freezer but made up of smaller cuts of pork.

Additional evidence was obtained to demonstrate that the resistance of trichinae in large cuts of pork to freezing at 0°F. was increased by a prior exposure to 35°F. (5.6°C) for a period of 150 days, and that some larvae so treated may survive at least 20 days in a 9 cubic foot freezer filled with 309 pounds of pork in large cuts. If such a freezer is overloaded by as much as 19 percent, trichinae in large cuts of fresh pork are killed in 20 days, but pork precooled for 179 days contained surviving larvae. If the freezer is overloaded by as much as 38 percent, trichinae may survive 20 days in fresh pork. In this instance, trichinae in precooled pork survived no more than 17 days at 0°F. (BPL) (ADP b2-15)

Investigations on trichinellosis are also being conducted under a PL 480 grant to the Polish Academy of Science, Warsaw, on the epidemiological, epizootiological, and immunological aspects of this disease to establish information on the incidence of Trichinella spiralis in people and domestic and wild animals throughout the country. Allergic tests for diagnosis of the disease are being assessed. Other studies indicate that the intestinal flora in the host's digestive tract may affect the invasive ability of the larvae. (Poland) (E21-ADP-9)

D. Intestinal Roundworm (Ascaris suum)

In work at the Nebraska Agricultural Experiment Station, Lincoln, under a cooperative agreement with the USDA, sows were orally immunized with repeated doses of infective eggs of Ascaris suum. Baby pigs from the immunized sows made highly significant average daily weight gains (ADG) when compared with baby pigs from nonimmunized sows.

Intraperitoneal injections of a lipid enzyme complex isolated from the intestinal tract of adult Ascaris suum elicited the development of protective immunity in mice against the migrating larvae of A. suum. This was manifested by a significant reduction in the number of larvae migrating to the lungs and a rise in the relative percent gamma globulin in the serum.

Leucine amino peptidase, isolated from the intestinal tract of adult A. suum, did not elicit an immunologic response in mice when the enzyme was placed in the drinking water. Apparently the addition of leucine amino peptidase to the drinking water resulted in the mice becoming more susceptible to parasitism as a significantly larger number of larvae were recovered from the liver and lungs of the mice in the enzyme group when mice were challenged with 20,000 infective eggs of A. suum.

Intraperitoneal injections of leucine amino peptidase, isolated from the intestinal tract of adult A. suum, elicited the development of protective immunity in pigs against the migrating larvae of A. suum. This was evidenced by a significant reduction in the number of larvae migrating to the lungs of immunized pigs and a significant elevation in serum gamma globulin.

Inorganic pyrophosphatase was isolated from second stage larvae of A. suum by ammonium sulfate fractionation and subsequent CM cellulose chromatography. The characteristics of the enzyme were determined.

Semipurified inorganic pyrophosphatase was isolated from second stage larvae of A. suum and compared with an enzyme obtained from adult A. suum. Tests indicated steriochemical continuity of pyrophosphatase during development of the parasite. The role of pyrophosphatase in the production of functional immunity was investigated by attempted hyperimmunization of rabbits with adjuvanted enzyme.

If the anthelmintic 2,2-dichlorovinyl dimethyl phosphate (DDVP) is present in the intestine of pigs when larvae of A. suum escape from the egg shell, the larvae will be killed. When administered orally, DDVP did not kill the tissue phase of the migrating larvae of A. suum.
(Lincoln, Nebraska) (ADP b2-12)

E. Strongyloides ransomi Infections in Baby Pigs

At the Regional Research Laboratory, Tifton, Georgia, researchers found that experimental infection of weaned pigs can severely affect their rate of gain and may lead to death of some pigs. Infected pigs fed an inadequate ration containing 14% crude protein did not gain as well as pigs fed an adequate ration containing 16% crude protein in the fall of 1963. When the experiment was repeated in the spring of 1964, no significant difference was observed between pigs on the two rations. The combined results show that S. ransomi causes a lowered rate of gain in infected pigs regardless of the ration fed, although the deleterious effects may be more severe in pigs fed an inadequate ration.

Experimental infection of Duroc and Hampshire pigs with S. ransomi reduced their naturally acquired Ascaris suum infection. Duroc pigs raised with Hampshire pigs had 6 times as many ascarids. These unsuspected variables apparently masked possible differences in the reaction of the two breeds to infection with S. ransomi. (Tifton, Georgia) (ADP b2-17)

F. Biochemical Aspects of Host-Parasite Relationship

At the Beltsville Parasitological Laboratory researchers report that females of the swine kidney worm, Stephanurus dentatus, and large roundworm of poultry, Ascaridia galli, had a much higher total lipid content than the males of these parasites. The total lipid content of the female swine kidney worm was 43.8 mg/gram of worm tissue; of the male 27.7 mg. Female Ascaridia from poultry yielded 25 mg/gram of worm tissue, whereas the males had only 12.9 mg. Females also had a larger percentage of neutral lipids than the males; 54.4 percent versus 22.05 percent in the kidney worm and 59.7 percent versus 28.4 percent in the poultry ascarid. These differences could be accounted for in the greater amount of glycerides in the neutral lipid fraction of the female worms. Since glycerides are sources of energy

the presence of relatively large amounts of these compounds in the female may be related to the functions of egg production and oviposition.
(BPL) (ADP b2-18)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Swine Kidneyworms

Shorb, D. A., and Shorb, M. S. 1964. Lipids of the male and female swine kidney worm, Stephanurus dentatus. J. Parasitol. 50(Sect. 2):56

Stewart, T. B., Hale, O. M., and Andrews, J. S. 1964. Eradication of the Swine Kidney Worm, Stephanurus dentatus, from Experimental Pastures by Herd Management. Amer. J. Vet. Res., 25:1141-1150.

Stewart, T. B., Smith, W. N., and Hale, O. M. 1964. Breed Differences in the Response of Swine to Infection with Strongyloides ransomi. Jour. An. Sci., 24:284.

Intestinal Roundworms

Kelley, George W., and Nayak, D. P. 1964. Acquired Immunity to Migrating Larvae of Ascaris suum Induced in Pigs by Repeated Oral Inoculations of Infective Eggs. J. Parasitol. 50:499-503.

Nayak, D. P., and Kelley, George W. 1965. Synergistic Effect of Ascaris Migration and Influenza in Mice. J. Parasitol. 51:297-298.

Nayak, D. P., Kelley, George W., and Underdahl, N. R. 1964. The Enhancing Effect of Swine Lungworms on Swine Influenza Infections. Cornell Vet., 54:160-175.

Rhodes, M. B., Marsh, C. L., and Kelley, George W. 1964. Studies in Helminth Enzymology. III. Malic Dehydrogenase of Ascaris suum. Exp. Parasitol., 15:403-409.

Rhodes, M. B., Nayak, D. P., Kelley, George W., and Marsh, C. L. 1965. Studies in Helminth Enzymology. IV. Immune Responses to Malin Dehydrogenase from Ascaris suum. Exp. Parasitol. 16:373-381.

AREA NO. 13 - PARASITES AND PARASITIC DISEASES OF SHEEP AND GOATS

Problem. The cost of parasitic diseases to the sheep and goat industry of the United States is estimated to be in excess of $45 million annually. Disorders caused by parasites are ubiquitous, generally insidious and often overlooked entirely. Diagnosis is difficult, and successful treatments for many of these diseases are not available. Moreover, management practices to avoid spread of parasitisms and to control them are often ineffectual. The problem is to develop, through a planned, balanced program of basic and applied research, knowledge for preventing, controlling or eradicating parasitic diseases so as to provide for healthy animals, insure adequate supplies of high quality lamb for an expanding population, avoid or minimize economic losses caused by these diseases, and thereby contribute to a prosperous agriculture, a sound national economy, a high standard of living, and a healthy population.

USDA AND COOPERATIVE PROGRAM

The Department has a continuous long-term program involving biochemists, parasitologists, and veterinarians engaged in both basic studies and the application of known principles to the solution of parasites and parasitic diseases of sheep and goats. Research is being conducted on these diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 6.7 professional man-years. This effort is divided among sub-headings as follows:

Life Cycles of Sheep Coccidial Parasites 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Gastrointestinal Nematodes 1.1 at the Beltsville Parasitological Laboratory, and under a cooperative agreement with the Kentucky Agricultural Experiment Station at Lexington.

Immunity to the Intestinal Worm, Trichostrongylus colubriformis 1.5 at the Regional Animal Disease Research Laboratory, Auburn, Alabama.

Biology, Pathogenesis, and Control of Helminth Parasites of Sheep in the Southwest 1.0 at the University Park, New Mexico, field station, and through informal cooperation with the New Mexico Agricultural Experiment Station at University Park.

Effect of Intestinal Roundworms on the Tensile Strength and Sulfur Content of Wool 0.1 under a cooperative agreement with the North Dakota Agricultural Experiment Station, Fargo.

Control of the Common Sheep Scab Mite 2.0 at the Parasite Research Laboratory, Albuquerque, New Mexico.

PROGRAM OF STATE EXPERIMENT STATIONS

Most of the research in this area by the States is concerned with sheep parasites. Work is closely interrelated with parasite research in cattle and much of the basic information derived is applicable to cattle, sheep and goats. Some of this work is coordinated through regional research (W-35) previously mentioned under Area No. 11.

Basic investigations are being carried out at a number of locations on the changes induced in the normal body processes of the host during varying degrees of parasitic infection. The mechanism of immunity which develops from exposure to parasites is being studied in detail to determine possible methods of immunization against the various species of sheep parasites.

Other studies are in progress to determine the effects on intensities of parasitic infections of climatic conditions, type and stage of plant growth, rate of pasture stocking, types of feed and other procedures of herd management.

The States have 9.6 professional man-years involved in sheep and goat parasite research.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Coccidial Parasites

Researchers at the Regional Animal Disease Laboratory, Auburn, Alabama, report the following work:

Eimeria ahsata, at 15 days postinoculation, has been found to have small, second generation schizonts with less than 48 merozoites. In addition, some of the second generation merozoites penetrate the nucleus of epithelial cells in intestinal glands and further development occurs inside the nucleus. This is the first discovery of an intranuclear stage in any of the coccidia of sheep, resembling the intranuclear stages of E. alabamensis in cattle, the first intranuclear development found in any coccidium of domestic animal.

Additional work was done on the life cycle of Eimeria intricata in sheep because of the availability of pure cultures resulting from isolation by micromanipulation. As a result of inoculating 6 lambs with large numbers of oocysts of E. intricata, 4 became infected with prepatent periods of each at 22 days post-inoculation (PI). The peaks of oocyst discharge ranged between 22 and 24 days PI and the patent periods lasted 2 to 6 days.

Sections of intestines of 4 lambs were processed and examined and, in addition, 4 more lambs were inoculated and sections made and examined. Data are reported at the following days PI: 2,6,8,12,15,16, 18, and 23 days. The greatest numbers of parasites were found progressively later in the life cycle, as was expected. All intermediate stages have now been found and

described. The most unusual findings are as follows: 1) the immature schizonts had a bur or "sunburst" appearance, due to radiating tips of merozoites leading outward from peripherally located nuclei. 2) the largest schizont was 104 by 32.5 μ and the schizonts were limited to the small intestine. They were not the macroscopic schizonts measuring up to 700 μ located in the abomasum, as has been thought for E. intricata. 3) later in the cycle, at 23 days, the parasites extended down into the cecum and upper colon. 4) last and most unusual, out of hundreds of parasitic stages examined, all except two were found in the crypts of Leiberkuhn, either freed in epithelial cells in the lumen or in the epithelial cells lining the glandular crypts, protruding into the lumen. This is a new endogenous site for coccidia of domestic animals and furnishes information for differential diagnoses during necropsies.

(Auburn, Alabama) (ADP b3-19)

B. Gastrointestinal Nematodes

Work was continued at the Beltsville Parasitological Laboratory for the sixth consecutive grazing season on the control of parasitism in sheep by means of pasture management combined with chemotherapy.

Also, data became available during the year from an experiment on the response of sheep to primary infection with the large stomach worm, Haemonchus contortus, in which experiment a member of the Beltsville Parasitological Laboratory participated while serving as a Fulbright Fellow at the C.S.I.R.O. Animal Health Laboratory, Sydney, Australia.

(BPL) (ADP b3-16)

In research studies under a cooperative agreement with the Kentucky Agricultural Experiment Station at Lexington, the earlier discovery of a strain of the common stomach worm of sheep tolerant to standard therapeutic doses of thiabendazole raises the serious question relative to its use-life in the practical application of parasite control in sheep. The common stomach worm is the most important parasite of sheep in the eastern United States, as well as other parts of the world, and a successful sheep operation is dependent on the control of this parasite.

The present strain became manifest after three drenches of thiabendazole at 4-week intervals which, under field conditions, could limit its effectiveness to less than one grazing season. Thiabendazole became widely used soon after its introduction because it was highly satisfactory and for the most part this has been the general experience. However, the discovery of the foregoing strain stressed the need to maintain surveillance and motivated the observations in the present report on three separate flocks in the central Kentucky region. In general the investigations revealed that a high degree of effectiveness was being maintained in two of the flocks and a slightly lesser degree obtained in the third flock. One of the organic phosphates was tested against the thiabendazole tolerant strain and found to be highly efficacious. (Lexington, Kentucky) (ADP b3-16)

C. Immunity to Trichostrongylus colubriformis

At the Division's Regional Research Laboratory, Auburn, Alabama, researchers determined, in two separate tests, that parasitic third-stage larvae (2-day-old infections) provided 81 and 84 percent protection against reinoculation with Trichostrongylus colubriformis, whereas all stages of development (12-day-old infections) provided 98 and 99 percent protection. The results showed that removal of immature worms did not interfere with development of acquired immunity by the host.

In earlier studies it was found that in guinea pigs immunized by single inoculation with 5,000 T. colubriformis that the worms of a challenge inoculation were reduced by 17, 73, and 98 percent 2, 5, and 12 days after the inoculation. An experiment was conducted to determine more specifically when the immune response exerts its maximum effect between 5 and 12 days.

Forty-five guinea pigs were each inoculated with 5,000 larvae, and all were given a therapeutic dose of thiabendazole 20 days later. Thirty-three days after initial inoculation, 5,000 larvae were administered to each of the immunized animals and to 45 controls. Five immunized and 5 control guinea pigs were killed 2, 5, 7, 8, 9, and 10 days after the challenge. Reduction of worms expressed in percentage based on ratio of average number of worms per immunized group to average number per control group were - 24, 56, 77, 86, 99, 99, respectively.

The results indicated that the bulk of the worms in the challenge were overwhelmed by the immune response while in the parasitic fourth-stage development, and that they were expelled from the host by the ninth day. In addition, the worms recovered on the ninth day were all in the fourth molt in the immunized animals, whereas about 40 percent were in the fifth stage in the controls. (Auburn, Alabama) (ADP b3-21)

D. Life Histories, Biology, Pathogenesis, and Control of Helminth Parasites of Sheep in the Southwest

At the Division's Research Laboratory at University Park, New Mexico, it was determined that few, if any, internal parasites of ruminants are as important in sheep and cattle production as Haemonchus contortus, the large stomach worm. This worm is responsible for extensive mortality and morbidity. Work is in progress at this laboratory to ascertain whether weakened strains of these parasites, collected from wild ruminants such as the pronghorn antelope, might be used successfully to render lambs immune to the ravages of Haemonchus. The results to date are promising enough to justify further intensive investigations along these lines. Efforts are now being concentrated on a combination of larval inoculation of lambs followed by treatment to remove the immunizing infections. Work now in progress is designed to determine the optimum number of larvae and the optimum time of treatment.

Wehrdikmansia cervipedis is a large worm parasite which occurs under the skin and adjacent areas of a high percentage of deer and other wild ruminants. It is closely related to several parasites known to occur in livestock. Work recently completed in the Southwest provides important new information about W. cervipedis, including the precise location of the larval stages in the host, a description of these larval stages, and an indication that the ears may be the chief site for the adult parasites. This information provides an additional and accurate means of diagnosing the infections.

In studies on the life history of the liver tapeworm of sheep, a parasite responsible for considerable loss due to liver condemnations, efforts were made to assess the importance of various factors which might play a part in failures to infect lambs experimentally. It was determined that 1) a range diet for test lambs may not be essential, 2) there is marked variation between species of psocids, the insect vector of the parasites, in their susceptibility to tapeworm infection, 3) age of psocids may be of little importance, and 4) eggs of the tapeworm remain infective for at least 6 days after recovery.

Nematodirus lanceolatus, a species of intestinal worm relatively new to the United States, has been found in an increasing number of ruminants in the Southwest. Preliminary studies on the life history of this parasite have provided a new means of specific diagnosis based on size of eggs in fecal samples. Information about time required for development and structural characteristics of the various stages has been assembled.

Because of the economic losses sustained by sheep producers from liver tapeworms and liver flukes, intensive efforts have been made to find satisfactory and effective treatments. Experiments completed at University Park several years ago resulted in the finding of the first compound shown to be capable of removing practically 100% of liver tapeworms. This compound is bithionel. Subsequently an additional effective compound - Bayer 2353 - was discovered. Although the latter compound was found to be ineffective against liver flukes, it has been demonstrated that bithionol is quite effective against the adults and probably against the usually drug-resistant immature stages. Thus, further work may demonstrate that bithionol, or compounds closely related, will have the exceptional properties of being effective against most, if not all stages of both liver tapeworms and liver flukes. (University Park, New Mexico) (ADP b3-17 and 18)

E. Effect of Gastrointestinal Nematodes in Lambs on the Sulfur Content and Tensile Strength of Wool

In a research study at the North Dakota Agricultural Experiment Station, Fargo, under a cooperative agreement with the USDA, 24 lambs that had been raised as parasite free as possible, were divided into 3 groups of 8 lambs each. One group was maintained as non-infected controls, one group was given 5,000 infective larvae of gastrointestinal nematodes of the trichostrongylus type per lamb, perorally by capsule, and the third group was

given 50,000 infective larvae per lamb. Wool was collected from an area over the shoulders, at the initiation of the study and again at the termination of the study. The tensile strength of the fibers and the sulfur content of the wool was determined. The change in sulfur content of the wool was apparently related to the level of infection of nematodes. The non-infected lambs had the largest increase in sulfur content and the heavily infected group had the least increase in sulfur in the wool. The low level of infection did not appear to influence the tensile strength of the wool fibers, but the high level of infection had an apparent adverse effect on the tensile strength. (Fargo, North Dakota) (ADP b3-20)

F. Control of the Common Sheep Scab Mite, Psoroptes ovis

The following work has been reported from the Division's Regional Laboratory at Albuquerque, New Mexico.

Dipping Vat Trials with New and Established Acaricides for the Control of Sheep Scabies. In an extensive series of tests with various established and candidate miticides involving 400 sheep heavily infested with Psoroptes ovis, the mite responsible for common sheep scab, only 0.06% lindane, 0.5% toxaphene, and 0.375% Co-Ral (Coumophos) effected 100% control of the mites following dipping. Unfortunately, the Co-Ral formulation was toxic to several of the sheep, which leaves only lindane and toxaphene as satisfactory therapeutic agents against sheep scabies. Other candidate compounds, including 0.3% Ciodrin, 0.1 and 0.15% Shell S.D. 4072, 0.2% Baytex (Tiguvon), and 0.06% Diazinon, proved unsuccessful in eliminating psoroptic scabies infestations.

The Residual Effectiveness of Candidate and Established Acaricides Against Challenge with Psoroptes ovis. Experiments were conducted in FY 1965 designed to determine the duration of effectiveness of candidate parasiticides on treated sheep, against challenge by scab mites on infested sheep. A total of approximately 430 sheep were involved in these tests; 250 of these consisted of an infested, or challenging flock, while 180 were clean, or uninfested sheep. Of the uninfested sheep, 20 were untreated and served as controls, while the remainder were distributed among 15 groups of 10 each, treated with 9 different chemical compounds.

Valuable information was elicited relative to the applicability of such tests to the evaluation of candidate miticides, the rapidity of spread of common scabies under various circumstances, the relative merits of scabicides when applied as baths versus dusts, and the duration of protection afforded by full fleeces as opposed to short wools, after dipping in select parasiticides.

The longest period of absolute protection against infestation (72 days) was afforded by 0.06% lindane bath, and the shortest (10 days) by 0.15% Shell S.D. 4072 dip. The first control subject became infested in 9 days after exposure.

New Approaches to the Administration of Candidate Chemotherapeutic Agents for the Control of Psoroptes ovis on Sheep. Tests designed to determine if methods other than dipping might be effective against Psoroptes ovis, the mite responsible for common sheep scab, were completed in FY 1965. Vapona collars (10% DDVP) worn by 10 heavily infested sheep for 2 months, 3% Ciodrin dust applied twice by means of a Howry-Burg apparatus to 38 sheep, and 2% Famphur feed additive (5 mg/kg/day) for 23 days to 5 principals, were all ineffective. (Albuquerque, New Mexico) (ADP b3-22)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Gastrointestinal Nematodes

Allen, R. W. 1964. Additional notes on parasites of bighorn sheep on the Desert Game Range, Nevada. Desert Bighorn Council 1964 Transactions, 8th Ann. Meet. April 7-9, Puerto San Felipe, Baja California, Mexico, pp.5-9.

Allen, R. W., and Erling, H. G. 1964. Parasites of bighorn sheep and mule deer in Arizona with new host records. J. Parasit. 50(Suppl.):71.

Bizzell, W. E., and Ciordia, Honorico. 1965. The course of uncomplicated experimental infections with Trichostrongylus colubriformis in calves. J. Parasit. 51:174.

Donald, A. D., Dineen, J. K., Turner, J. H., and Wagland, B. M. 1964. The dynamics of the host-parasite relationship. I. Nematodirus spathiger infection in sheep. Parasitology 54:527-544.

Drudge, J. H., Szanto, Joseph, Wyant, Z. N., and Elam, George. 1964. Field studies on parasite control in sheep: Comparison of thiabendazole, ruelene, and phenothiazine. Amer. J. Vet. Res., 25:1512-1518.

Scabies

Roberts, I. H., and Meleney, W. P. 1965. Psorergatic Acariasis in Cattle. Jour. AVMA, 146:17-23.

Roberts, I. H., Meleney, W. P., and Colbenson, H. P. 1965. Psorergatic Acariasis on a New Mexico Range Ewe. Jour. AVMA, 146:24-29.

AREA NO. 14 - PARASITES AND PARASITIC DISEASES OF POULTRY

Problem. Parasites and parasitic diseases probably cost the poultry industry many millions of dollars annually by causing intestinal disturbances, emaciation, retarded growth, reduced egg production, and deaths. Parasites are ubiquitous, many times insidious, and often overlooked until birds are damaged irreparably. Early diagnosis is difficult, and reliable treatments for many devastating parasitoses are not available. Moreover, some management practices, intended to avoid spread of parasites and to control them, have been found ineffectual as is shown by the increasing importance of certain parasites in broiler production. The problem is to develop, through a planned, balanced program of basic and applied research, methods for preventing, controlling or eradicating parasitic diseases, thus affording economical production of healthy poultry and sound products in supplies adequate to meet the needs of an expanding population.

USDA AND COOPERATIVE PROGRAM

The Department has a continuous long-term program involving parasitologists, biologists, and chemists, engaged in both basic studies and the application of known principles to the solution of the problem of parasites and parasitic diseases of poultry.

The Federal scientific effort devoted to research in this area totals 4.0 professional man-years. This effort is applied as follows:

Control of Coccidiosis 2.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Biology of Nematode Parasites 1.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Biological investigations of Protozoan Parasites and Parasitic Diseases, with Special Reference to those of the Gastrointestinal Tract 1.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

PROGRAM OF STATE EXPERIMENT STATIONS

The major emphasis in this area is being placed on the problem of coccidiosis. The interaction of nutrition, bacterial organisms and coccidia in bringing about disease outbreaks is being investigated. The effectiveness of coccidial vaccines is under evaluation and factors are being determined which influence the immunity obtained from these vaccines. Micro-environmental conditions favoring development of infective coccidial oocysts are being studied. The problem of coccidiosis in turkeys is under evaluation and the important species involved in outbreaks are being determined.

Several States have research in progress on blackhead to develop improved means for controlling this parasite.

There are 5.8 professional man-years allocated to research on poultry parasites at the States.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. The Biology of the Nematode Parasites of Poultry and Related Birds with Special Reference to the Application of Findings to Control Measures.

At the Beltsville Parasitological Laboratory recent experimental work confirmed previously reported findings that the drug, thiabendazole, was very effective when used to remove poultry gapeworms from young turkeys. In three experiments, mash containing 0.5 percent of thiabendazole by weight was fed ad libitum for periods of 17, 17, and 20 days beginning on the day of experimental infection and on the 28th and 30th day post-infection, respectively. All of the worms were removed from the 33 treated birds. Twenty untreated controls harbored 247 pairs of gapeworms. Possible systemic action of the drug was indicated by its action against worms that had already reached the trachea before treatment was initiated. No adverse effects attributable to the drug were noted. (BPL) (ADP b4-10)

B. Biological investigations of Parasites and Parasitic Diseases of Poultry with Special Reference to those of the Gastrointestinal Tract.

Through research at the Beltsville Parasitological Laboratory, an effective means has been developed for detecting contamination of soil in poultry yards, gamebird runs, and turkey ranges with cecal worms and the protozoan parasite that causes blackhead. Earthworms, preferably the common fishing worm, Lumbricus, collected at appropriate times from such soil, are fed to birds susceptible to the parasites sought, and to blackhead. Evaluations are based on 1) the occurrence of blackhead in birds so fed; 2) the occurrence of cecal worms in them, and 3) the presence of Histomonas, ascertained by post-mortem examinations, by ante-mortem fecal examinations, and clinical observations. From predetermined values based on prior testing, the extent of contamination of the test plots, and the interval since such contamination occurred can be approximated.

Histomonas meleagridis can be attenuated by serial in vitro passage, causing it to lose its virulence and invasive power, but still retain much of its ability to immunize chickens when inoculated into them, rectally. To a lesser extent, this is also true of turkeys. With improved methods, this could have practical applications, at least with some breeds or strains of birds.

Critical studies of stages in the life cycle of one of the poultry coccidia, Eimeria acervulina, in its host, the chicken, uncovered several new facts. There are 3, and possibly 4, generations of merozoites produced in the

endogenous life cycle of Eimeria acervulina. The first two generations develop deep within the glands of Lieberkühn. Their development is exceedingly quick. Mature schizonts of the 2nd generation are present at 48 hours and immature schizonts of the 1st generation are not present before 30 hours. This means that two generations are produced in 18 hours. The 3rd generation develops superficially in the villar epithelium. A 4th generation, morphologically similar to the 3rd, is most probable. Assuming only 3 generations and using suitable equations to calculate the theoretical yield, it was found that the actual oocyst yield from a given dosage of oocysts was really more than the theoretical. Assuming the more logical 4 generations, the actual oocyst yield from the same dosage was 30-40% lower than the theoretical - a level it should be at assuming a substantial number of sporozoites from the dosage are lost in the host.

After emerging from the oocysts in the intestine of the chicken, the sporozoites enter the tips of the intestinal villi and pass into the lamina propria, or core, of the villus. Within the lamina propria, they are engulfed by macrophages and taken to the duodenal glands of Lieberkühn. The macrophages serve as a defense mechanism against infection as well as a transport system for the sporozoites. Most of the sporozoites are either ejected into the gland lumen or destroyed by the macrophages. The number destined to develop to 1st generation schizonts most probably depends on the inherent potential of the oocyst culture from which they come and on the quantity and/or quality of cell stimulus received. No 1st generation schizonts were found after 48 hours. It is possible that the schizogonous cycle, like the alarm on a clock, is "timed" to "go off" when the proper host cell stimulus is provided and the production of 1st generation schizonts stops when the upper threshold of the inherent potential is reached.

(BPL) (ADP b4-11)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Biology of the Nematode Parasite of Poultry

Hwang, J. C. 1964. Hemogram of turkey poults experimentally infected with Syngamus trachea. Avian Diseases, 8:380-390.

Wehr, E. E. 1964. Anthelmintic activity of thiabendazole against the gapeworm, Syngamus trachea, in turkeys. J. Parasitol., 50:60.

Biological Investigations of Protozoan Parasites

Lund, Everett E. 1964. Biological Control of Animal Parasites. Symposium, Pest Control by Chemical, Biological, Genetic, and Physical Means, Montreal

Coccidiosis of Poultry

Doran, David J., and Farr, Marion M. 1965. Susceptibility of 1- and 3-day-old chicks to infection with the coccidium, Eimeria acervulina. J. Protozool. 12(2):160-166.

AREA NO. 15 - TREATMENT FOR REMOVAL OF PARASITES OF DOMESTIC ANIMALS

Problem. Parasites of food animals are responsible for losses to livestock producers approximating a billion dollars annually. This estimate, moreover, is conservative since it does not take into account costs of treatment and other control measures. Chemical antiparasitic agents are the most powerful weapons presently available against parasites and the diseases they cause, yet specific treatments generally have a comparatively short period of usefulness. Many of the currently preferred treatments were unknown a decade or so ago and, in all probability few, if any of those in use today will be primary choices a decade or so hence. Moreover, the growing concern with respect to residues in edible tissues and organs of treated animals and birds necessitates development of control measures other than treatment. The problem is to develop, through a planned, balanced program of basic and applied research control methods that minimize reliance on extrinsic chemicals. These include investigations of immunological procedures, management practices which minimize exposure of animals to parasitic infections, and natural control agents such as parasites, pathogenic microorganisms, and predators of economically important livestock pests.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving veterinarians, parasitologists, pharmacologists, and biochemists engaged in both basic studies and the application of known principles in developing treatments for removal or control of parasites of domestic animals. Research is being conducted on this problem at the following designated locations.

The Federal scientific effort devoted to research in this area totals 9.5 professional man-years. This effort is applied as follows:

Chemical Control of Parasitic Diseases 1.5 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

New and Improved Anthelmintics 3.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Hazards of Residues from Treatment for Parasites 3.5 at the Regional Animal Disease Research Laboratory, Auburn, Alabama.

Pathobiology of Parasitic Infections 1.0 at the Albuquerque, New Mexico, field station.

Control and Eradication of Scabies 0.5 at the Albuquerque, New Mexico, field station.

PROGRAM OF STATE EXPERIMENT STATIONS

State research in this area is designed to provide detailed information needed for the safe and effective application of chemicals in the control of livestock parasites. Promising new compounds are evaluated singly and in various combinations to determine effective treatment against the important species of livestock parasites. Major emphasis is being placed on treatment of infections due to nematodes. Other work is directed toward therapeutic control of coccidia, histomonads, liver flukes and tapeworms.

Methods of administering anthelmintic compounds and dosages required for the most effective parasite control are evaluated along with considerations of toxic effects which the drugs may have on host animals. Potentially useful compounds are being compared for effectiveness as measured by reductions in parasites and parasite eggs and increases in weight gains and feed efficiency in the treated host. Simplified methods for administering anthelmintics on a herd or flock basis are being developed and the use of these compounds is being coordinated with research on effective management methods.

Basic research at several locations is seeking fundamental information on how anthelmintics act upon biochemical systems involved in parasite metabolism. The problem of drug resistance in certain strains of parasites also is under study.

The States are allocating 15.2 professional man-years to research on the treatment of livestock parasites.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Investigations of dimetridazole and other potential chemotherapeutic agents as treatments for bovine veneral trichomoniasis.

In research work at the Beltsville Parasitological Laboratory, Trichomonas foetus infections were eliminated from 6 bulls by the oral administration of dimetridazole at the rate of 50 mg/kg of body weight daily for 5 consecutive days. The chemical was given either by capsule or admixed with the feed. Five daily doses at 25 mg/kg administered by capsule freed another bull of T. foetus, but this level was ineffective when fed in grain to 2 bulls. One of the latter subsequently became negative after repeated courses of treatment at higher dosages. The other bull retained the infection.

Research efforts with dimetridazole during the current year have been concentrated on the development of a safe and reliable treatment involving a single administration of the chemical. Trichomonads have not been recovered on repeated examinations of 5 bulls that were each given only one intravenous injection. Two other bulls required a second treatment at a higher dosage level before they became negative for T. foetus. The chemical is well tolerated at all dosage levels. (BPL) (ADP b5-19)

B. Evaluation, development, and standardization of chemical methods of established or reported value for the control of parasitic diseases of livestock and poultry.

Researchers at the Beltsville Parasitological Laboratory report that nihydrazone, at a level of 0.011% in the feed of chickens with experimental Eimeria tenella infections, greatly reduced mortality and oocyst production. However, the incidence of cecal lesions was high and the growth of infected birds was poor. A similar level of medication did not inhibit the growth of uninfected birds.

Strains of Eimeria tenella, passed through 58 successive groups of chickens medicated with Unistat or arsenosobenzene, respectively, developed a pronounced tolerance to the specific drugs. The degree of tolerance was greater in the Unistat-medicated strain than in the one exposed to arsenosobenzene.

A strain of E. tenella serially exposed to amprolium for 48 generations developed a distinct tolerance to the chemical. Beginning with the 39th passage, there were occasional deaths among the infected, medicated birds.

Unsporulated oocysts of E. tenella were not adversely affected by storage at refrigerator temperatures for 3 months. The rate of sporulation and virulence were comparable to those of conventional cultures.

Dimetridazole administered in the drinking water for 3 days, eliminated Trichomonas gallinae infections, the cause of canker in pigeons.

Piperazines are excellent drugs for removal of the large roundworm, Ascaridia galli, from chickens, but they proved to be only moderately effective against Ascaridia columbae of pigeons. Dose rates equal to or higher than those that are ordinarily very effective against the chicken ascarid failed to achieve comparable efficacy against the large roundworm in pigeons. The drugs stupify the worms and their removal is effected by intestinal peristalsis. Accordingly, if peristalsis is impeded for any reason, the effects of the drugs may disappear before the worms can be expelled. Because the intestine of the chicken is much larger than that of the pigeon, interference with peristalsis may be less likely to occur. This may account, at least in part, for the difference in efficiency of piperazines in the two hosts.

Limited tests with subcutaneous injections of methyridine, a new systemic anthelmintic, indicate that this chemical may compare favorably with other trichuricides for dogs. A dosage of 150 mg/kg of body weight was completely effective against 348 Trichuris in 4 dogs, and at 200 mg/kg the drug removed all of 385 Trichuris from 6 dogs. The larger dosage was presumably fully effective also in 5 additional dogs that were not necropsied.

Moderate activity was exhibited against Ancylostoma in limited trials at the 200-mg level. Anthelmintic action against Toxocara, Taenia, and

Dipylidium was either negligible or too limited to permit even provisional interpretations of efficacy.

Emesis, ataxia, soft feces, and acute irritation at the site of injection were evidenced at all dosage levels. These reactions, however, were transitory. (BPL) (ADP b5-5)

Field trials in Mississippi, conducted by researchers at the Regional Animal Disease Laboratory, Auburn, Alabama, indicated that Thibenzole was an effective anthelmintic in lambs when administered monthly in the feed. At a dose rate of 44 mg/kg of body weight, Thibenzole controlled nematode parasites of lambs as well as drenchings with phenothiazine with the exception of Haemonchus contortus. The ease of administration of Thibenzole in this manner, eliminating the need for individual treatment, would favor its use, especially in areas where H. contortus is not of primary concern.
(Auburn, Alabama) (ADP b5-5(Rev.)

C. Investigations to develop new and improved chemical agents for the treatment, prevention, or control of helminthic parasites in farm animals.

At the Beltsville Parasitological Laboratory, researchers found that an 0.5% thiabendazole-medicated mash was remarkably effective in removing the large intestinal roundworm, Ascaridia columbae, from experimentally infected pigeons. Medicated mash was fed ad libitum for approximately 1 to 2 weeks, and the treatment removed, in the aggregate, 95% (1,522) of 1,592 worms from 57 birds. The drug exhibited anthelmintic action against both immature and mature worms. This is the first satisfactory treatment developed for the removal of Ascaridia columbae from pigeons.

Limited tests with subcutaneous injections of methyridine, a new systemic anthelmintic, indicate that this chemical may compare favorably with other trichuricides for dogs. A dosage of 150 mg/kg of body weight was completely effective against 348 Trichuris in 4 dogs, and at 200 mg/kg, the drug removed all of 385 Trichuris from 6 dogs. The larger dosage was presumably fully effective also in 5 additional dogs that were not necropsied.

Moderate activity was exhibited against Ancylostoma in limited trials at the 200-mg level. Anthelmintic action against Toxocara, Taenia, and Dipylidium was either negligible or too limited to permit even provisional interpretations of efficacy.

Emesis, ataxia, soft feces, and acute irritation at the site of injection were evidenced at all dosage levels. These reactions, however, were transitory.

In limited trials, methyridine, a systemic anthelmintic, exhibited marked action against the intestinal capillarid, Capillaria obsignata, in pigeons. Doses of 45 mg, administered subcutaneously, removed all of 1,390 Capillaria from 10 birds. Doses of 35 mg were about equally effective, removing 99%

of 1,667 capillarids from 6 birds. A slight swelling at the site of injection and a temporary incoordination were the only untoward signs observed.

Capillaria obsignata has been responsible for outbreaks of capillariasis among chickens, particularly laying hens, in the United States as well as in other countries. The parasite also occurs in turkeys and peafowl.
(BPL) (ADP b5-18)

D. Control of Internal Parasites of Livestock by Management Practices that will not Create Consumer Residue Hazards.

At the Experiment, Georgia, substation of the Regional Research Laboratory, Auburn, Alabama, a study was continued on the epidemiology of helminthiasis in sheep in Georgia. So far, September appears to be the most critical month for grazing pastures, as evidenced by the larger number of eggs passed during that month. Judging from egg counts, younger animals were more heavily parasitized than older ones. (Auburn, Alabama) (ADP b5-16)

E. Pathobiology of Parasitic Infections with Special Reference to the Injuriousness of Arthropod Parasites, and the Economic Gain and Efficiency of Control Measures.

At the Regional Research Laboratory, Albuquerque, New Mexico, work was directed toward the screening of candidate parasiticides and investigations into methods of administration for the control of lice on cattle. Livestock pesticides are most efficient, and are most widely accepted by stockmen, when they are easily applied and destroy several parasites rather than merely one. In a search for compounds and methods of administration applicable to the simultaneous control of cattle grubs, horn flies, and lice, several materials were evaluated from the standpoint of their lousicidal effectiveness on cattle. Famphur, administered orally in the feed, daily, proved 100% effective in destroying sucking lice on cattle, but did not eliminate biting lice, and was completely ineffective against scab mites. Collars and belts impregnated with Vapona proved 100% effective in eliminating both sucking and biting lice from cattle. Compound SD 8447, applied as a pour-on to the backs of cattle, was not effective in controlling lice. Further work with Famphur and Vapona against lice on cattle is strongly indicated.
(Albuquerque, New Mexico) (ADP b5-13)

F. Development of New Approaches and Methods for the Control and Eradication of Scabies in Sheep and Cattle.

At the Regional Research Laboratory, Albuquerque, New Mexico, continued observations were made on the comparative pathogenicity of various strains of Psoroptes ovis. Completed aspects of studies on sheep, involving various field strains of Psoroptes ovis, the mite responsible for common scabies of sheep, cattle, and horses, show conclusively that differences in virulence or aggressiveness among strains are profound. Such virulent

strains are not only highly pathogenic to sheep, but show evidence of resistance to drugs as well. The practical contribution of these investigations has been to provide material for the perpetuation of a dependable source of infested hosts in various stages of severe clinical parasitism, suitable for the screening and evaluation of candidate and established acaricides. Furthermore, maintenance of discrete strains broadened old concepts regarding clinical manifestations of psoroptic acariasis, while inbreeding and outcrossing of strains may lead to advances in the field of arthropod parasite strain exhaustion and host-resistance.

Psorergatic acariasis was discovered on a Hereford cow in New Mexico in 1963. Subsequently, 14 other cases from 7 different premises in New Mexico and Texas were found. Attempts to transfer Psorergates bos from the original infested cow to other cattle, and to white rats and rabbits, were unsuccessful. Gross clinical manifestations of acariasis have not been associated with the presence of the mites. There is reason to believe Psorergates bos to be a widely distributed parasite of cattle which, due to its small size and apparent inoffensiveness to its host, has heretofore escaped detection.

Psorergates ovis, the Australian itch mite, was found on a New Mexico range ewe. The infestation was characterized by alopecia and broken, matted fleece. Mites in all stages of development were found in numerous skin scrapings but could not be found by histologic examination of skin sections. Efforts to transmit the mites from the ewe to rabbits and white rats were unsuccessful. The infestation was, however, successfully transmitted to 2 unshorn lambs in close confinement with the unshorn subject for 6 to 8 months. After all sheep involved were shorn, transmission to a ewe required less than 4 months.

Subsequently, the natural transference of the parasite to 4 additional sheep was accomplished, but efforts to infest goats and calves, through contact, failed. Since the mite does transfer from sheep to sheep without inordinate difficulty, because it was found in a Southwestern range flock not otherwise remarkable, and since its discovery was the third reported in the United States, it is possible that its distribution may be more widespread than is currently suspected.

In work on the blood cell responses in the sheep to Psoroptic scabies, studies on the blood of non-scabby sheep, which are not otherwise heavily parasitized, such as range sheep commonly encountered in the Southwest, indicate that lymphocytes and neutrophils, the most numerous white cells, appear in almost equal numbers, while monocytes outnumber eosinophiles as much as 10:1. However, when these sheep are exposed to common scab, the eosinophiles outnumber the monocytes even before clinical symptoms of scab are detectable. As the disease progresses, the eosinophiles come to outnumber the monocytes as much as 30:1 or more. No predictable change in the relative numbers of lymphocytes and neutrophils were observed.

In observations on the host-parasite relationship of Psoroptes ovis to sheep, it was found that when sheep infested with scab mites are isolated from other flock members, the parasites appear invariably to die, and the sheep spontaneously recover from scabies. Certain phases of the study of this phenomenon, completed in fiscal year 1965, suggest that the virulence or disease-producing capacity of the mites involved, and the status of clinical infestation at the time of isolation, have no bearing on the duration of survival of mite populations. The reason for mite population extinction is as yet unknown, but now appears to be more closely associated with host response than with parasite strain exhaustion.

(Albuquerque, New Mexico) (ADP b5-15)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Bovine Veneral Trichomoniasis

McLoughlin, D. K. 1964. Activity of dimetridazole in Trichomonas foetus infections. J. Parasit., 50:2:57.

Control of parasitic diseases of livestock and poultry

Baird, D. M., White, P. E., Ciordia, H., Bizzell, W. E., and McCampbell, H.C. 1964. Low-level medicated mineral for the control of horn flies and cattle grubs. Georgia Agr. Exp. Stations, Mimeo series N.S. 205, 9pp.

McLoughlin, D. K., and Gardiner, J. L. 1965. The activity of nihydrazone in Eimeria tenella infections - Laboratory trials. Avian Diseases 9:21-23.

AREA 16 - MISCELLANEOUS PARASITES AND PARASITIC DISEASES

Problem. Parasitism is a way of life that characterizes the majority of living things. Except for basic life processes, it is probably the commonest biological phenomenon. More than 50,000 kinds of animal parasites (i.e., parasites classified as animals as opposed to those classified as plants) are known. New varieties are being discovered and described at a rate of about 500 per year. Some devastating parasites, indigenous to foreign countries, threaten to surmount barriers imposed against them. Certain of these have already gained new footholds in livestock, poultry, and wildlife. Essential elements of procedure against parasites--established, exotic, or new--are accurate diagnosis, development of full knowledge about them, and research on effective control measures. The primary requirement is development through research of up-to-date knowledge of classification and identification supported by a complete reference collection of parasites, including type specimens and familiarity with global research already done. Basic investigations of parasitisms as biological phenomena are involved, especially in host-parasite relations, immunology, serology, ultrastructure, and other aspects of diagnosis and control. The problem is to develop and maintain up-to-date methods of identification and the essential, supporting reference collections, as well as complete parasitological information extracted from the world's scientific literature; investigate important phenomena and host-parasite systems not covered in specific host categories; and provide bases for detection and control that are adequate to meet existing and anticipated needs, through research on problems involving various parasites and hosts, including wild animals and birds important to agriculture.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program for parasitologists, biochemists, and microbiologists, engaged in basic and applied research in this area. Research is being conducted on the following problems at the designated locations.

The Federal scientific effort devoted to research in this area totals 10.5 professional man-years. This effort is divided among subheadings as follows:

Maintenance and publication of author, subject, and host index-catalogues 2.5 at the Beltsville Parasitological Laboratory.

Immunologic and other biologic approaches to the prevention and control of parasitic diseases 3.0 at the Beltsville Parasitological Laboratory.

Chemical and physical elements of parasites and parasite-host relationship 2.0 at the Beltsville Parasitological Laboratory.

Taxonomic investigations of parasites 2.0 at the Beltsville Parasitological Laboratory.

Maintenance of parasite collection 1.0 at the Beltsville Parasitological Laboratory.

PROGRAM OF STATE EXPERIMENT STATIONS

Information is being compiled at a number of States on the incidence and importance of specific parasites of livestock. Several States have work dealing with the morphology and comparative anatomy of parasites to aid in identification and classification of the species involved. Research is in progress at several locations on the laboratory culture of adult parasitic nematodes. Basic information is being obtained on the nutritional requirements of these parasites and the products of metabolism are being evaluated as potential immunizing agents.

The States have 7.5 professional man-years allocated to this area.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Maintenance and Publication of Author, Parasite-Subject, Host, and Anthelmintic Catalogues.

At the Division's Beltsville Parasitological Laboratory, the Index-Catalogue of Medical and Veterinary Zoology has been maintained and expanded in its various sections - Author, Parasite-Subject, and Host Catalogues, and Checklist of Specific and Subspecific Names. New entries augmenting the Catalogues are as follows: Author entries, 8,672; Parasite-subject entries, 24,873 (including 22,560 Parasite entries, 2,313 Anthelmintic entries); and Host entries, 6,303. The Index-Catalogue has continued to supply references for the Anthelmintic Catalogue of the Anti-parasitic Investigations. Literature on plant parasitic nematodes was fully indexed for about the first half of the year - since that time only taxonomic papers on this group have been indexed.

New genera and species of parasites are as follows: Protozoa: 7 n.g., 149 n. sp.; Trematoda: 36 n.g., 145 n. sp.; Cestoda: 6 n.g., 47 n. sp.; Nematoda: 85 n.g., 607 n. sp.; Arthropoda and miscellaneous groups: 23 n. g., 373 n. sp. There have been 204 new citations of periodicals added to the Catalogue. An average of 500 periodicals are examined each day at the National Agricultural Library for parasitological papers to be indexed for the Index-Catalogue.

The Index-Catalogue has had more than 40 visitors from the United States and eight other countries, some of them staying several days and consulting it as a source of information. (ADP b6-14)

Supplement 14, Authors: A to Z, was published in September, 1964, and Supplement 15, Authors: A to Z, was published in March, 1965. Material for the Protozoa and Trematoda parts of the Parasite-Subject Section of Supplement 15 is being processed for photo-offset printing. Part 3: Supergenera and Genera D of Subjects: Trematoda and Trematode Diseases, was published in the fall of 1964. (BPL) (ADP b6-9)

B. Immunologic and other biologic approaches to the prevention and control of parasitic diseases.

At the Beltsville Parasitological Laboratory, attempts to separate and concentrate antibodies to Stephanurus dentatus by gradual thawing of frozen serum during centrifugation were partly successful. Antibodies to excretory gland extract were concentrated 4-fold in fractions containing the heavier serum protein components. Antibodies to intestinal extract were present in approximately equal concentrations in all fractions.

Studies were continued on the in vitro growth of Stephanurus dentatus in an attempt to extend development beyond the fourth stage and to evaluate various ingredients. The findings were evaluated by comparing the results with data previously obtained using medium PB-1 enriched with NCTC-109. The data showed that vitamin B-12 was essential to the development of S. dentatus. In the absence of this factor, living deformed larvae occurred in the third and fourth stages. Although it was shown previously that serum was essential for development to the fourth stage, new data showed that a serum concentration of 50% was more beneficial than one of only 12.5 percent. Greater yields of fourth stage S. dentatus were obtained in KW-1, a modification of PB-109 formulated by omitting the balanced salt solution and increasing the serum to 50 percent. Development of S. dentatus was not enhanced by the addition of sterilized, heat-dried, meat-egg medium, meat-egg-phytone, and liver extracts, and suspensions of fresh, trypsinized swine kidney and liver cells to cultures of the parasite developing in KW-1. However, evidence was obtained that fourth stage larvae actively feed on particles of meat-egg and on kidney and liver cells.

Studies were continued on the in vitro growth of Oesophagostomum radiatum in an attempt to extend development beyond the fourth molt and to evaluate various ingredients. Experiments designed to study the effect of environmental conditions showed that O. radiatum larvae developed in 59 days to young adults, a stage not previously attained, when grown in screw-capped Erlenmeyer flasks containing 20 ml of medium SM-1 and incubated in an upright, stationary position at 38.5 C. In earlier studies, it was shown that the larvae developed only to fourth molt when cultured in screw-capped tubes containing 3 ml of SM-1 and incubated in a horizontal, stationary position. Using the same protocols, the effects of free gas exchange (cotton-plugged flasks) and anaerobiasis (5% CO_2 -95% N_2) were studied. Both of these environmental conditions inhibited development to the fourth stage and caused a death rate of 95% of the total inoculum by the 34th day in the cotton-plugged flasks and by the 38th day in the anaerobic flasks.

Development comparable to that obtained in cultures maintained in screw-capped flasks was attained when cotton-plugged and anaerobic cultures were changed to screw-capped vessels. Evidence also showed that after several transfers under anaerobic conditions, cultures removed to screw-capped flasks underwent synchronous development to each advanced stage.

In the nutritive trials, the addition of autoclaved suspensions of hemoglobin, egg-meat medium, and gastric mucosa to medium SM-1 containing larval cultures in all stages up to fourth molt resulted in the death of larvae in fourth stage and fourth molt and inhibited development of larvae in parasitic third stage and third molt.

Studies on the in vitro growth of Haemonchus contortus in medium SM-1 were continued in an attempt to extend development beyond the fourth molt by changing the environmental cultivation protocols. The findings were evaluated by comparing the results with data previously obtained by the tube-method. Using screw-capped flasks, H. contortus advanced to the fifth stage or young adults in 19 days. Although survival could not be extended beyond the 40th day, advancement to fifth stage represents significant progress since this stage had not been previously attained.

A first attempt has been made to grow Oesophagostomum columbianum through its parasitic stages. Cultured with H. contortus in screw-capped flasks containing SM-1, O. columbianum developed through early to late parasitic third stage, third molt, early and late fourth stages and fourth molt as early as 4, 11, 11, 18, and 35 days in culture, respectively. After 35 days, larvae in all stages appeared starved.

Oral vaccination with infective larvae of Dictyocaulus filaria of sheep was tested as a means for the protection of calves against infection with the cattle lungworm, D. viviparus. In three small-scale exploratory trials, five calves were vaccinated and five served as controls. Dosages of D. filaria larvae, numbers of vaccinations, intervals between final vaccination and challenge, and levels of challenge, differed between trials. Each of the controls acquired a substantial patent D. viviparus infection from a challenge dose of larvae, whereas four of the five comparably exposed vaccinated individuals were highly resistant or immune to the establishment of mature D. viviparus. D. filaria apparently did not mature in the infected calves. The smallest dosage per vaccination given to young calves was 5,000 larvae. It was too pathogenic for use for immunization in early calfhood. Short yearlings tolerated repeated vaccination with about 2,000 larvae very well. (Beltsville, Maryland) ADP b6-10)

C. Chemical and Physical Elements of Parasites and Parasite-host Relationship.

At the Beltsville Parasitological Laboratory, extracts from the excretory glands of swine kidney worms and serums from kidney-worm-infected and control swine were fractionated by density-gradient ultracentrifugation. When the

extracts are layered on a sucrose solution which increases in concentration from top to bottom and the whole is subjected to prolonged (18 hours) centrifuging at ultra speeds, the antigenic components of the extract are distributed from near the top to about three-fourths of the way down the column of fluid. The reactive antibodies of serum under the same treatment are distributed about half way down the column. The antibodies from control swine react with antigens which sediment only slightly, while the antibodies from the infected swine react with antigens over the whole range.

Considerable volumes (3 - 15 mls.) of solutions of kidney worm antigens of improved specificity were prepared. This was done by collecting and concentrating those antigenic components of a mixture which migrate toward the positive electrode of the "barrier electrophoresis" apparatus.

Comparison of the red pigment of gapeworm with hemoglobin from the host birds, turkeys, reveals that the two pigments differ in their sedimentation properties in the ultracentrifuge, their migration rates in an electric field, and in their susceptibility to denaturation by strongly alkaline solutions or by the drug barium antimonyl tartrate. The worm pigment contains the same percentage of iron as does hemoglobin.

(Beltsville, Maryland) (ADP b6-11)

D. Taxonomic investigations of Helminths and other parasites.

Three manuscripts were prepared for publication. A new species of hookworm was described from specimens recovered from the small intestine of deer in Louisiana and the description is supplemented with a key to the seven species in the hookworm genus Monodontus. A new genus and a new species of a spiruroid nematode were described from specimens recovered from the stomach of peccaries in Texas and New Mexico, and a new species of Nematodirus was described from specimens collected from the small intestine of mountain goats in Montana and Alberta. (BPL) (ADP b6-12)

At the Beltsville Parasitological Laboratory, 317 lots of specimens were identified (protozoans 1, trematodes 12, cestodes 7, acanthocephalans 3, nematodes 173, and arthropods 121). Among these were numerous parasites of medical and veterinary importance. One lot of nematodes recovered from the intestine of a human patient in the Philippines represents a new species and the first case of intestinal parasitism by a species of Capillaria in man. A mature male meningeal worm, Odocolleostrongylus tenuis, a nematode previously reported in the brain and spinal cord of sheep, deer, and moose in North America, was found in a pituitary gland removed from a deer in Georgia. Th

The following parasites were collected from animals and items offered for entry into the United States: (Ticks) Amblyomma cajennense, A. dissimile, A. gemma, D. variegatum, Boophilus decoloratus, B. microplus, Dermacentor sp. probably D. everstianus, D. nigrolineatus, D. nitens, D. parumapertus, Hyalomma anatolicum, H. marginatum, Ixodes hexagonus, I. scapularis,

Ornithodoros megnini, Rhipicephalus bursa, R. evertsi, and R. pulchellus; (Lice) Haematopinus eurysternus and Linognathus africanus; (Flies) Hippobosca sp. and Melophagus ovinus. (Beltsville, Maryland) (ADP b6-16)

E. Maintenance of Parasite Collection.

At the Beltsville Parasitological Laboratory, 731 lots of specimens (protozoans 1, trematodes 164, cestodes 50, acanthocephalans 12, nematodes 339, arthropods 139, and miscellaneous 16) were added to the parasite collection. (BPL) (ADP b6-15)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Maintenance and Publication of Index-Catalogues

Doss, Mildred A., Roach, Kathryn F., and Breen, Virginia L. 1964. Index-Catalogue of Medical and Veterinary Zoology, Subjects: Trematoda and Trematode Diseases, Supergenera and Genera D. U. S. Government Printing Office.

Humphrey, Judith M., and Segal, Dorothy B. 1964. Index-Catalogue of Medical and Veterinary Zoology, Supplement 14, Authors: A-Z. U. S. Government Printing Office.

Humphrey, Judith M., and Segal, Dorothy B. 1965. Index-Catalogue of Medical and Veterinary Zoology, Supplement 15, Authors: A-Z. U. S. Government Printing Office.

Immunologic Approaches to Parasitic Diseases.

Lucker, J. T., Vegors, H. H., and Douvres, F. W. 1964. Immunization against the Cattle Lungworm: Oral vaccination with infective Dictyocaulus filaria larvae. Proc. Helm. Soc. Wash., 31:153-158.

Rose, J. E., Baisden, L. A., and Tromba, F. G. 1964. Ultracentrifugal Fractionation of Reactants in a Gel-diffusion Precipitin Technique in Stephanuriasis. J. Parasit., 50:504-508.

Tromba, F. G. 1965. Biological control of Helminthic Diseases. Vet. Med., 60:69-74.

Taxonomic Investigations.

Becklund, W. W. 1964. Revised check list of Internal and External Parasites of Domestic Animals in the United States and Possessions and in Canada. Amer. J. Vet. Res., 25:1380-1416.

Line Project Check List - Reporting Year July 1, 1964 to June 30, 1965

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project inc. in Summary of Progress (Yes)(No)	Area and Subheading
ADP a1	Infectious and Non-Infectious Diseases of Cattle			
ADP a1-3(Rev.)	Brucellosis of cattle	*Ames, Iowa	Yes	1-A
		St. Paul, Minnesota	Yes	1-A
		Wooster, Ohio	Yes	1-A
		Madison, Wisconsin	Yes	1-A
ADP a1-9(Rev.2)	Vibriosis of Cattle	Ames, Iowa	No	
		Ithaca, New York	Yes	1-B
ADP a1-13(Rev.)	Tuberculosis of Cattle	Ames, Iowa	Yes	1-C
		East Lansing, Mich.	Yes	1-C
ADP a1-14(c) (Rev.)	Mucosal-Respiratory Disease-Complex of Cattle	Ames, Iowa	Yes	1-D
		Ft. Collins, Colo.	Yes	1-D
		Lafayette, Indiana	Yes	1-D
	Bovine Virus Diarrhea	Ames, Iowa	Yes	1-D
ADP a1-15(R)	Mastitis of Cattle	Ames, Iowa	Yes	1-E
		Davis, California	Yes	1-E
ADP 1-17	Respiratory Diseases of Cattle (Shipping Fever)	Ames, Iowa	Yes	1-F
ADP a1-19	Infertility in Cattle other than by Vibriosis and Trichomoniasis	Ames, Iowa	Yes	1-G
ADP a1-21	Epizootic Bovine Abortion	Ames, Iowa	No	
		Davis, California	Yes	1-H
ADP a1-22	Foot Rot (Infectious Pododermatitis) of Cattle	Ames, Iowa	No	
ADP a1-24	Etiological, cytological and histochemic studies of Pulmonary Adenomatosis in Cattle	Ames, Iowa	No	
ADP a1-25	Immunization against Bovine Leptospirosis	Ames, Iowa	Yes	1-I
ADP a1-26	Chemotherapy in Leptospirosis	Ames, Iowa	Yes	1-J
ADP a1-29(C)	Enteritis in Young Calves	**Moscow, Idaho	No	
ADP a1-30	Bovine Lymphosarcoma	Ames, Iowa	Yes	1-K
ADP a1-32	Characterization and Classification of members of the genus Brucella	**Ames, Iowa	No	
ADP a1-35	Paratuberculosis of Cattle (Johne's Disease)	Ames, Iowa	Yes	1-L
ADP a1-37	Pink-Eye (Infectious Keratitis) of Cattle	Ames, Iowa	Yes	1-M
	The Immunizing Effect of Brucella Cell Wall (PL 480 (A10-ADP-6)	Jerusalem, Israel	Yes	1-A

* Completed during reporting period

** Initiated during reporting period

Line Project Check List -- Reporting Year July 1, 1964 to June 30, 1965

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project inc. in Summary of Progress (Yes)	(No)	Area and Subheading
ADP a2	Infectious and Noninfectious Diseases of Swine				
ADP a2-8(Rev.)	Studies on the causative agent (or agents), mode of spread, diagnosis, and control of atrophic rhinitis in swine	Ames, Iowa	Yes		2-B
ADP a2-10(Rev.)	Transmissible Gastroenteritis (TGE)	Ames, Iowa	Yes		2-C
		Davis, California	Yes		2-C
		Lafayette, Indiana	Yes		2-C
ADP a2-13(Rev.)	Pilot field studies to evaluate diagnostic tests, biologic products, and quarantine measures for a hog cholera eradication program	Live Oak, Florida	Yes		2-A-5
ADP a2-15	Erysipelas of swine	Ames, Iowa	Yes		2-D
		*Pulawy, Poland (PL 480 Grant) (E21-ADP-8)	No		
ADP a2-16	Brucellosis of Swine	Ames, Iowa	Yes		2-E
ADP a2-17(C)	Hog Cholera	Ames, Iowa	Yes		2-A-1,2, 3,4
		Urbana, Illinois	Yes		2-A-2
		Lincoln, Nebraska	Yes		2-A-2
ADP a2-18	*Infectious causes of infertility in swine other than brucellosis and leptospirosis	Ames, Iowa	No		
ADP a2-19	Abscesses in Swine	Ames, Iowa	Yes		2-F

*Terminated during reporting year

Line Project Check List -- Reporting Year July 1, 1964 to June 30, 1965

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project inc. in Summary of Progress (Yes)	(No)	Area and Subheading
ADP a3	Infectious and Non-Infectious Diseases of Sheep and Goats				
ADP a3-1(Rev.)	Vibriosis of Sheep	Ames, Iowa		No	
		Fort Collins, Colo.	Yes		3-B
		Bozeman, Montana	Yes		3-B
		Logan, Utah	Yes		3-B
ADP a3-3	Scrapie of Sheep	Compton, England (PL 480 E29-ADP-2)	Yes		3-C
		Edinburgh, Scotland (PL 480 E29-ADP-3)	Yes		3-C
ADP a3-4	Viral Ulcerative Dermatosis of Sheep	Fort Collins, Colo.	Yes		3-E
ADP a3-5	Bluetongue in sheep - diagnosis, transmission and control	Denver, Colorado	Yes		3-A
ADP a3-6	Paratuberculosis (Johne's Disease) of Sheep and Goats	Ames, Iowa	Yes		3-D
ADP a3-7	*Toxicological Effects of Oxalate-Containing Plants	Logan, Utah	Yes		3-F
ADP a3-8	*Identification of Teratogenic Agent in Veratrum californicum	Logan, Utah	Yes		3-G
ADP a3-9	*Chronic Toxicity of Herbicide Accumulation in Sheep Tissues	Logan, Utah	Yes		3-H
ADP a3-10	*Persistence and Transmission of Viral and Rickettsial Diseases in Helminths	Pullman, Washington	Yes		6-C

*Initiated during reporting period

Line Project Check List -- Reporting Year July 1, 1964 to June 30, 1965

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project inc. in Summary of Progress (Yes)	(No)	Area and Subheading
ADP a4	Diseases and Parasites of Horses				
ADP b6-13(C)	Investigations on the serological diagnosis, transmission, and control of equine piroplasmosis	Beltsville, Maryland Gainesville, Florida Lexington, Kentucky	Yes Yes Yes		4-A
	Study of the horsesickness virus	Ankara, Turkey PL 480 A22-ADP-7	Yes		4-A
	Gastrophilus pseudo-hemorrhoidalis (equine parasite)	Ankara, Turkey PL 480 a22-ADP-4	Yes		4-A

Line Project Check List -- Reporting Year July 1, 1964 to June 30, 1965

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project inc. in Summary of Progress (Yes)	(No)	Area and Subheading
ADP a5	Investigations of Infectious and Non-Infectious Diseases of Poultry				
ADP a5-2(R)	Salmonellosis of Poultry	Athens, Georgia	Yes		5-C
ADP a7-25	Investigations of the Genus Pasteurella	Ames, Iowa	Yes		5-D
ADP a5-17	Chronic Respiratory Disease Complex in Chickens and Turkeys	Ames, Iowa	Yes		5-B
		Storrs, Connecticut	Yes		5-B
		Newark, Delaware	Yes		5-B
		Athens, Georgia	Yes		5-B
		Amherst, Massachusetts	Yes		5-B
		Ithaca, New York	Yes		5-B
		Raleigh, North Carolina	Yes		5-B
		College Station, Texas	Yes		5-B
		Blacksburg, Virginia	Yes		5-B
		St. Paul, Minnesota	Yes		5-B
		Jerusalum, Israel (AlO-ADP-9)	No		
ADP a5-18	Newcastle Disease	Athens, Georgia	Yes		5-B
		Ames, Iowa	Yes		5-E
		Orono, Maine	Yes		5-E
		Madison, Wisconsin	Yes		5-E
		Pulawy, Poland (E21-ADP-2)(E21-ADP-6)	No		
ADP a5-20	Ornithosis in Poultry	Davis, California	Yes		5-A
		St. Paul, Minnesota	Yes		5-A
		Corvallis, Oregon	Yes		5-A
		College Station, Texas	Yes		5-A
		Ames, Iowa	Yes		5-A
ADP a5-21	Turkey Airsacculitis	Ames, Iowa	Yes		5-B
		St. Paul, Minnesota	Yes		5-B
		Madison, Wisconsin	Yes		5-B
ADP a5-23	Infectious Bronchitis in Poultry	Athens, Georgia	Yes		5-B
		Ames, Iowa	Yes		5-F
	Fowl Plague	Madrid, Spain (E25-ADP-1)	No		
ADP a5-27	*Avian Encephalomyelitis	Ames, Iowa	Yes		5-G

*Initiated during reporting year

Line Project Check List -- Reporting Year July 1, 1964 to June 30, 1965

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project inc. in Summary of Progress (Yes) (No)	Area and Subheading
ADP a6	Infectious and Non-Infectious Diseases of Fur Animals, including Rabbits			
ADP a6-5	Enteric Disease Complex of Rabbits	Fontana, California	Yes	6-A
ADP a6-6	Respiratory Disease Complex of Rabbits	Fontana, California	Yes	6-A
ADP a6-7	Field and Laboratory Studies of Diseases of Fur Animals	Pullman, Washington	Yes	6-B
ADP a6-8	*Studies on the Persistence and Transmission of viral and rickettsial diseases in helminths associated with diseases of fur animals	Pullman, Washington	Yes	6-C

*Superseded during reporting year by ADP a3-10

Line Project Check List -- Reporting Year July 1, 1964 to June 30, 1965

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project inc. in Summary of Progress (Yes) (No)	Area and Subheading
ADP a7	Miscellaneous Infectious and Non-infectious Diseases of Animals			
ADP a7-5(Rev.)	**Reservoirs, transmission and immunological studies of vesicular stomatitis	Ames, Iowa	No	
ADP a7-7(Rev.) Includes ADP a1-28)	**Investigation of livestock poisoning by plants, their toxicity for different classes of livestock and methods of treatment and prevention	Logan, Utah Ames, Iowa Sao Paulo, Brazil (PL 480 (S3-ADP-5)	Yes Yes No	7-G 7-G
ADP a7-8(Rev.)	**Investigation of the toxicity of herbicides and herbicide-treated plants to livestock	Logan, Utah	Yes	3-H
ADP a7-12(Rev.)	**Use of radioactive isotopes in studying insecticide toxicology in animals	Kerrville, Texas	No	
ADP a7-14(Rev.)	Fractionation, purification and characterization of the components of normal and immune sera of animals	Ames, Iowa	Yes	7-A
ADP a7-15	**Bloat in ruminants	Ames, Iowa Davis, California College Park, Md. State College, Miss. Ithaca, New York Madison, Wisconsin	Yes No Yes No Yes Yes	7-K 7-B 7-B 7-B
ADP a7-16(Rev.)	Preparedness for Laboratory Assistance in Diagnosis of Foreign Animal Diseases	Greenport, Long Island, New York	No	
ADP a7-17	Studies to develop alleviators and diagnostic tests for plant poisoning and methods to avoid harmful residues in animal tissues from ingesting chemically treated plants	Logan, Utah	Yes	7-H
ADP a7-18	Investigations in cattle and sheep of the biochemical effects of agricultural chemicals and control substances	Kerrville, Texas Nacogdoches, Texas	Yes Yes	7-D 7-D
ADP a7-19	Detoxication mechanisms in cattle and sheep	Kerrville, Texas	Yes	7-E
ADP a7-20	Characterization of cytological responses to toxic actions of antiparasitic and other agricultural chemicals in cattle and sheep tissues	Kerrville, Texas	Yes	7-F
ADP a7-21	**Susceptibility of wild animals to foot-and-mouth disease	Greenport, Long Island, New York	No	

continued on next page

Line Project Check List - Area 7, continued

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project inc. in Summary of Progress (Yes)(No)	Area and Subheading
ADP a7-22	Studies of the incidence and pathology of cancer and other tumors in food-producing animals	Ankara, Turkey (PL 480 A22-ADP-2)	No	
ADP a7-23	Toxicological and pathological effects of insecticides, herbicides, fungicides, and other agricultural chemicals on livestock and poultry	Kerrville, Texas	Yes	7-C
ADP a7-24	Mycotic Diseases of Domestic Animals	Ames, Iowa	Yes	7-I
ADP a7-25	Investigations of the Genus Pasteurella	Ames, Iowa	Yes	5-D
ADP a7-26	Biological Changes Associated with Neuropathological Conditions in Animals	Ames, Iowa	Yes	7-J
ADP a7-27	Physiopathological Investigations of the Interrelations between the Respiratory, Circulatory, and Digestive Systems of Animals	Ames, Iowa	Yes	7-K
ADP a7-28	Proteins and Other Complex Molecules from Animal Disease Agents Derived Primarily from Surface Structures and Extracellular Products	Ames, Iowa	Yes	7-L
ADP a7-29	Chemical and Physical Studies on Microbial Antigens	Ames, Iowa	Yes	7-M
ADP a7-30	*Microbiology of the Ruminant Digestive Tract and Its Relation to Digestive Disturbances	Ames, Iowa	Yes	7-N
ADP a7-31	Physiology of Normal Mammalian Cells Grown in Tissue Cultures	Ames, Iowa	No	

*Initiated during reporting year
**Discontinued during reporting year

Line Project Check List - Reporting Year July 1, 1964 to June 30, 1965

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project inc. in Summary of Progress (Yes)(No)	Area and Subheading
ADP a8	Foot-and-Mouth and Other Exotic Diseases of Cattle			
ADP a8-8(R)	Immunological investigations - Studies on foot-and-mouth disease virus	Greenport, Long Island New York	Yes	8 - A
ADP a8-10(R)	Immunological investigations to determine the mechanism of antibody formation using viruses of exotic animal diseases	"	Yes	8 - B
ADP a8-11(R)	Immune response to various types and sub-types of foot-and-mouth disease virus	"	Yes	8 - C
ADP a8-12(R)	Development of methods for production of large quantities of foot-and-mouth disease virus by tissue culture methods	"	Yes	8 - D
ADP a8-14(R)	Establishment and characterization of cell lines and cell strains for the propagation of foot-and-mouth and other exotic disease agents of cattle	"	Yes	8 - E
ADP a8-17(R)	Mechanism of the interaction between foot-and-mouth disease virus molecules and host cells	"	Yes	8 - F
ADP a8-18(R)	Investigations of the genetic biochemistry of foot-and-mouth disease virus	"	Yes	8 - G
ADP a8-19(R)	Effects of certain chemical and physical environments on foot-mouth-disease virus	"	Yes	8 - H
ADP a8-20(R)	Bulk freeze-drying of foot-and-mouth disease virus, vaccines, and antiserums	"	Yes	8 - I
ADP a8-24	*The Survival and Transmission of foot-and-mouth disease virus in the semen of susceptible species of animals	"	No	
ADP a8-25	Identification, purification, and chemical and physical characterization of foot-and-mouth disease virus and other exotic animal viruses	"	Yes	8 - J
ADP a8-26	Immuno-chemical investigations of foot-and-mouth disease	"	Yes	8 - K
ADP a8-27	Microbiological Investigations - Attenuation of representative types of foot-and-mouth disease virus	"	No	

Continued on next page

Line Project Check List - Area 8 continued

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project inc. in Summary of Progress (Yes)(No)	Area and Subheading
ADP a8-28	*Survival and inactivation of foot-and-mouth disease virus in meat and meat by-products	Greenport, Long Island New York	Yes	8 - L
ADP a8-29	Studies on the biological mechanisms of natural resistance and susceptibility of foot-and-mouth disease virus	"	Yes	8 - M
ADP a8-30	Biological alterations of foot-and-mouth disease virus from continued residence in cell cultures	"	Yes	8 - N
ADP a8-31	Morphologic Aspects of Virus-cell Relationships	"	Yes	8 - O
ADP a8-32	Diagnostic and Immunizing procedures for Contagious Bovine Pleuropneumonia	"	Yes	8 - P
	Studies on foot-and-mouth disease (E3-ADP-2)	Sao Paulo, Brazil (PL 480 Grant)	Yes	8 - Q
	Studies of various indigenous types of foot-and-mouth disease virus, and the production of a vaccine for the control of FMD in Turkey (A22-ADP-8)	Etlik, Turkey (PL 480 Grant)	Yes	8 - R

* Completed during reporting period

Line Project Check List -- Reporting Year July 1, 1964 to June 30, 1965

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project inc. in Summary of Progress (Yes) (No)	Area and Subheading
ADP a9	Foot-and-Mouth and Other Exotic Diseases of Swine			
ADP a9-1(Rev.)	Immunological investigations of foot-and-mouth disease of swine	Greenport, L. I. New York	Yes	9-A
ADP a9-2(Rev.)	Investigations of African Swine Fever	Greenport, L. I. New York	Yes	9-B
		Kenya, East Africa	Yes	9-B
		Madrid, Spain	Yes	9-B
		PL 480 E25-ADP-4		

Line Project Check List -- Reporting Year July 1, 1964 to June 30, 1965

Work and Line Project Number	Work and Line Project Titles	During Past Year	Line project inc. in Summary of Progress (Yes) (No)	Area and Subheading
ADP all	Foot-and-Mouth and Other Exotic Diseases of Sheep			
ADP all-1	Immunological Investigations of Foot-and-Mouth Disease in Sheep	Greenport, L.I., New York	Yes	10-A
	Vaccine against Sheep Pox	Ankara, Turkey PL 480 A22-AD P-6	No	
		Madras, India PL 480 A7-ADP-5	No	

Line Project Check List -- Reporting Year July 1, 1964 to June 30, 1965

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line project inc. in Summary of: Progress (Yes)	Progress (No)	Area and Subheading
ADP bl	Parasites and Parasitic Diseases of Cattle				
ADP bl-6(Rev.)	*Etiological factors influencing gastro-intestinal nematodes of cattle	Auburn, Alabama Experiment, Georgia	No Yes		 11-A
ADP bl-12(R)	Effects of pasture mixtures and pasture on control of internal parasites	Auburn, Alabama Experiment, Georgia	No No		
ADP bl-19(R)	Acquisition and effects of roundworm parasites of cattle as influenced by diet	Beltsville, Maryland	No		
ADP bl-23(R)	Host-parasite relationship of coccidial parasites of cattle	Auburn, Alabama	Yes		11-B
ADP bl-24	Ecology and immunology of the cattle lungworm, Dictyocaulus viviparus	Beltsville, Maryland	No		
ADP bl-25	Clinical and physiological aspects of roundworm parasitism in cattle including anthelmintic treatment	Davis, California	Yes		11-C
ADP bl-26	Investigations of Trichomonad parasites	Logan, Utah Logan, Utah (Univ.)	Yes Yes		11-D 11-D
ADP bl-27	Host-parasite relationship of intestinal worms, Cooperia species, in cattle	Auburn, Alabama	Yes		11-E
ADP bl-28	Epizootiological-ecological investigations of the internal parasites of grazing cattle	Beltsville, Maryland	Yes		11-F
ADP bl-29	Etiology and immune response of cattle to winter coccidiosis	Logan, Utah Logan, Utah (Univ.) Bozeman, Montana	Yes Yes No		11-G 11-G
ADP bl-30	Anaplasmosis of cattle	Beltsville, Maryland	Yes		11-H
ADP bl-31	Interrelationship of diet and parasitic infection in the production of cattle	Auburn, Alabama	No		
ADP bl-32	Histochemistry of gastrointestinal nematodes of cattle	Auburn, Alabama	Yes		11-I
ADP bl-33	Parasites of cattle, with emphasis on Stephanofilarial species	University Park, New Mexico	Yes		11-J
ADP bl-34	**Effect of stocking rate and rotational grazing on internal parasitism of cattle	Auburn, Alabama Experiment, Georgia	Yes Yes		11-K 11-K

continued on next page

Line project check list - Area 11 continued

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line project inc. in Summary of Progress (Yes) (no)	Area and Subheading
	Environmental factors influencing parasites and parasitic diseases of economical importance in ruminants (cattle, sheep, and alpacas)	Lima, Peru (PL 480 S8-ADP-1)	Yes	11-L
	Anaplasmosis, piroplasmosis, and Babesiellosis of cattle	Montevideo, Uruguay (PL 480 S9-ADP-1)	Yes	11-M

* Completed during reporting year

** Initiated during reporting year

Line Project Check List -- Reporting Year July 1, 1964 to June 30, 1965

Work and Line Project Number	Work and Line Project Titles	work Locations During Past Year	Line project inc. in Summary of Progress (Yes) (No)	Area and Subheading
ADP b2	Parasites and parasitic diseases of swine			
ADP b2-4(Rev.)	*The effect of anthelmintic treatment on rate of gain when administered to parasitized pigs of different ages and on different nutrition levels	Tifton, Georgia	No	
ADP b2-10(Rev.)	Investigation of the bionomics and pathogenicity of the swine whipworm	Beltsville, Maryland	Yes	12-A
ADP b2-11(Rev.)	Control of swine kidney worms by herd management, etc.	Raleigh, North Carolina	Yes	12-B
ADP b2-12(Rev.)	Investigations of the swine intestinal roundworm, Ascaris suum	Lincoln, Nebraska	Yes	12-D
ADP b2-15	Investigations of strains of Trichinella spiralis resistant to heat and cold and modes of transmission of the parasite	Beltsville, Maryland Warsaw, Poland PL 480 E21-ADP-9	Yes Yes	12-C 12-C
ADP b2-17	Studies of Strongyloides ransomi infections in baby pigs	Tifton, Georgia	Yes	12-E
ADP b2-18	Evaluation of biochemical and other aspects of the host-parasite relationship in the development and severity of helminthiases of swine	Beltsville, Maryland	Yes	12-F

*Discontinued during the reporting year

Line Project Check List -- Reporting Year July 1, 1964 to June 30, 1965

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project inc. in Summary of Progress (Yes) (No)	Area and Subheading
ADP b3	Parasites and Parasitic Diseases of Sheep and Goats			
ADP b3-15	*Investigations on the effects of helminthic infections on serum proteins of sheep and goats	Beltsville, Maryland	No	
ADP b3-16	Investigations of gastrointestinal nematodes and nematodiases of sheep and goats and measures for their control	Beltsville, Maryland Lexington, Kentucky	Yes Yes	13-B 13-B
ADP b3-17	The biology of the liver fluke, Fasciola hepatica, of sheep and cattle, etc.	University Park, New Mexico Ankara, Turkey PL 480 A22-ADP-1	Yes No	13-D
ADP b3-18	The life histories, biology, pathogenesis and control of several helminth parasites of sheep occurring in the Southwest	University Park, New Mexico	Yes	13-D
ADP b3-19	Studies on the life cycles of Eimeria ahasta and Eimeria crandallis, pathogenic coccidia of sheep	Auburn, Alabama	Yes	13-A
ADP b3-20	The effect of gastrointestinal nematodes on the tensile strength and sulfur content of wool	Fargo, North Dakota	Yes	13-E
ADP b3-21	Immunity to the intestinal worm, Trichostrongylus colubriformis, a parasite of ruminants	Auburn, Alabama	Yes	13-C
ADP b3-22	Control of the common sheep scab mite, Psoroptes ovis	Albuquerque, New Mexico	Yes	13-F

*Discontinued during reporting year

Line Project Check List -- Reporting Year July 1, 1964 to June 30, 1965

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project inc. in Summary of Progress (Yes) (No)	Area and Subheading
ADP b4	Parasites and Parasitic Diseases of Poultry			
ADP b4-9	Investigations for Controlling Coccidiosis of Poultry	Beltsville, Maryland	No	
ADP b4-10	The Biology of the Nematode Parasite of Poultry and related birds with Special Reference to the Application of Findings to Control Measures	"	Yes	14-A
ADP b4-11	Biological Investigations of Protozoan Parasites and Parasitic Diseases of Poultry, with Special Reference to those of the Gastrointestinal Tract	"	Yes	14-B

Line Project Check List -- Reporting Year July 1, 1964 to June 30, 1965

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project Summary of Progress (Yes)	(No)	Line Project inc. in Area and Subheading
ADP b5	Treatments for Removal or Control of Parasites of Domestic Animals				
ADP b5-5(Rev.)	Evaluation, development, and standardization of chemical methods of established or reported value for the control of parasitic diseases of livestock and poultry	Beltsville, Maryland Auburn, Alabama	Yes Yes		15-A 15-B
ADP b5-9(Rev.)	Investigations for Treatments for bovine venereal trichomoniasis	Beltsville, Maryland	Yes		15-A
ADP b5-12	Investigations of parasitic and related skin diseases of cattle, sheep, and swine, with primary emphasis on chemical control and basic biology of mange and scabies	Albuquerque, New Mexico	No		
ADP b5-13	Pathobiology of parasitic infections with special reference to the injuriousness of arthropod parasites, and the economic gain and efficiency of control measures	Albuquerque, New Mexico	Yes		15-E
ADP b5-14	Development of new methods for the control and eradication of ticks of domestic animals, with special reference to the cattle fever ticks, Boophilus annulatus and B. microplus, the principal vectors of bovine piroplasmosis	Albuquerque, New Mexico	No		
ADP b5-15	Development of new approaches and methods for the control and eradication of scabies in sheep and cattle	Albuquerque, New Mexico	Yes		15-F
ADP b5-16	Control of internal parasites of livestock by management practices that will not create consumer residue hazards	Auburn, Alabama	Yes		15-D
ADP b5-17	Investigations of antiparasitic agents and measures for the control of parasites belonging to the family Oestridae	Albuquerque, New Mexico	No		
ADP b5-18	Investigations to develop new and improved chemical agents for the treatment, prevention, or control of helminthic parasites in farm animals	Beltsville, Maryland	Yes		15-C

Line Project Check List -- Reporting Year July 1, 1964 to June 30, 1965

Work and Line Project Numbers	Work and Line Project Titles	Work Locations During Past Year	Line project inc. in Summary of: Progress (Yes)	(No)	Area and Subheading
ADP b6	Miscellaneous Parasites and Parasitic Diseases				
ADP b6-9(Rev.)	Publication of author, subject (parasite) and host index-catalogues of medical and veterinary zoology	Beltsville, Maryland	Yes		16-A
ADP b6-10	Investigation of immunologic and other biologic approaches to the prevention and control of parasitic diseases	"	Yes		16-B
ADP b6-11	Studies of the chemical and physical elements of parasites and parasite-host relationships in animals	"	Yes		16-C
ADP b6-12	Taxonomic Investigations of Helminths and Other parasites	"	Yes		16-D
ADP b6-13(C)	Equine Piroplasmosis	Beltville, Maryland Gainesville, Florida Lexington, Kentucky	Yes Yes Yes		4-A 4-A 4-A
ADP b6-14	Maintenance of Author, Parasite-Subject, Host, and Anthelmintic Catalogues and Checklist of Specific and Subspecific Names	Beltsville, Maryland	Yes		16-A
ADP b6-15	Maintenance of Parasite Collections	"	Yes		16-E
ADP b6-16	Identification of Parasites of Importance in Parasitological Research, Regulatory, and Quarantine, and Other Work	"	Yes		16-D


U.S. DEPARTMENT OF AGRICULTURE
NATIONAL AGRICULTURAL LIBRARY
UNITED STATES DEPARTMENT OF AGRICULTURE